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VOL. 33 MAY, 1948 No. 5

The Journal of Laboratory and Clinical Medicine

The Official Publication of the Central Society for Clinical Research

Clinical and Experimental

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Vol. 33, No. 5, May, 1948. The Journal of Laboratory and Clinical Medicine is published monthly by The C. V. Mosby Company, 3207 Washington Blvd., St. Louis (3), Mo. Subscription Price: United States, its Possessions, Pan-American Countries, $8.50 per year; Canada, $9.50; Foreign, $9.50. Subscriptions to medical students, interns and residents, half price. Entered as Second-Class Matter October 8, 1915, at the Post Office at St. Louis, Missouri, under Act of March 3, 1879. Printed in the U. S. A.

PENICILLIN IN THE TREATMENT OF ACTINOMYCOSIS

DONALD R. NICHOLS, M.D., AND WALLACE E. HERRELL, M.D.
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IN 1943 Florey and Florey[1] reported the results of the treatment with penicillin of two patients who had actinomycosis. In these patients penicillin appeared to have no effect on the infection. However, in the light of our present knowledge the amounts of penicillin used and the method of administration employed appear to have been inadequate. In 1943 the Committee on Chemotherapeutic and Other Agents of the National Research Council[2] also reported data on the use of penicillin in three patients who had actinomycosis. One patient improved and two died. No details as to the type of case or the method or duration of treatment were given. In 1944, in collaboration with Heilman, we[3] reported our initial observations on the use of penicillin in the treatment of twelve patients who had actinomycosis. No conclusions could be reached from these limited observations because the patients had been followed for inadequate periods. Treatment with penicillin of small groups of patients who had actinomycosis has been reported by other investigators.[4-9] The results have been encouraging.

Up to the present time, sixty patients who had actinomycosis have been treated with penicillin under our supervision. Fourteen of these patients have not been followed for a sufficient length of time to enable us to evaluate the results of treatment. Our present report, therefore, deals with our observations on the use of penicillin in the treatment of forty-six patients who had actinomycosis. These forty-six patients have been followed for periods of from one to five years since the conclusion of the treatment.

Considerable confusion and difference of opinion exist concerning the definition of the term actinomycosis. For the purposes of our investigative work we have confined our studies to infections caused by the micro-aerophilic organism *Actinomyces bovis*. Infections caused by the several species of the genus Nocardia have been classified separately and are not included in this report. The diagnosis of actinomycosis in each of our patients was made by direct examination or culture of pus obtained from a draining sinus or from material obtained at operation. All strains of *Actinomyces bovis* cultured were found to be sensitive to penicillin in vitro, the organism being inhibited by 0.01 to 0.1 unit of penicillin per cubic centimeter of culture medium.

In attempting to evaluate methods of treatment it is of importance to qualify carefully the general term actinomycosis, for the prognosis varies greatly according to the location of the lesion, the duration of the infection, and the

From the Division of Medicine, Mayo Clinic.
Read at the Meeting of the Central Society for Clinical Research, Chicago, Ill., Oct. 31, 1947.
Received for publication, Jan. 26, 1948.

general condition of the patient. A method of treatment which is effective when the soft tissues of the face and neck are involved is often entirely ineffective when actinomycosis involves other parts of the body. If the disease has been present sufficiently long to permit an overgrowth of fibrous tissue or an impairment of the general health of the patient, effective treatment of any type will be much more difficult. A classification of actinomycosis according to the organ or organs involved is the most desirable. However, inasmuch as the pathologic process and the prognosis are similar when certain regions of the body are invaded, for purposes of discussion a more general classification can be adopted. Our patients, therefore, have been grouped under five general headings: cervicofacial actinomycosis, pulmonary actinomycosis, abdominal actinomycosis, pelvic actinomycosis, and actinomycosis involving other parts of the body.

Of the group of forty-six patients on whom we are reporting data, twenty-six were suffering from cervicofacial actinomycosis, nine from pulmonary actinomycosis, eight from abdominal actinomycosis, and three from pelvic actinomycosis. Penicillin was administered to each of these patients either by intermittent intramuscular injections every three hours or by the continuous intravenous drip method. The dosages of penicillin varied widely between 80,000 and 1,000,000 units daily. In most cases penicillin was administered continuously for periods ranging from two to seven weeks. In some instances the penicillin was administered in courses of ten days each, with intervals of one to several weeks intervening.

RESULTS ACCORDING TO LOCATION

Cervicofacial Actinomycosis.—Twenty-six patients with cervicofacial actinomycosis received penicillin. Since 1940, twenty-five other patients with cervicofacial actinomycosis have been seen by our colleagues or by ourselves. These patients did not receive penicillin but were treated by other methods. The number of patients who recovered was appproximately equal in both of these groups, that is, in excess of 90 per cent (Table I). The significant difference between the two groups, however, appears to be the duration of treatment necessary to bring about recovery. When penicillin was used, satisfactory results were obtained after an average period of treatment of less than two months. When penicillin was not used, it was necessary to continue the treatment, on an average, for nearly six months. These figures include the entire period from the onset of treatment to the discontinuance of all forms of therapy. In both groups treatment was carried out continuously in some cases and at varying intervals in others.

TABLE I. PENICILLIN THERAPY FOR CERVICOFACIAL ACTINOMYCOSIS

TREATMENT	NUMBER OF CASES	RECOVERY		FAILURE		AVERAGE DURATION OF TREATMENT (MO.)
		NUMBER	PER CENT	NUMBER	PER CENT	
Penicillin	26	24	92	2	8	1.8
No penicillin	25	24	96	1	4	5.9

Pulmonary Actinomycosis.—The most striking results of treatment with penicillin occurred in the patients who had pulmonary, abdominal, or pelvic actinomycosis, since patients with these types of actinomycosis had uniformly poor prognoses when other types of treatment were used. Nine patients who had pulmonary actinomycosis received penicillin (Table II). In this group there were five recoveries and four failures. Since 1940, thirteen other patients who had pulmonary actinomycosis have been seen by our colleagues or ourselves. None of these patients received penicillin, but they were treated by other methods. Only one of them appears to have recovered.

TABLE II. PENICILLIN THERAPY FOR PULMONARY ACTINOMYCOSIS

TREATMENT	NUMBER OF CASES	RECOVERY		FAILURE	
		NUMBER	PER CENT	NUMBER	PER CENT
Penicillin	9	5	56	4	44
No penicillin	13	1	8	12	92

Abdominal and Pelvic Actinomycosis.—Eight patients who had abdominal actinomycosis and three patients who had pelvic actinomycosis received penicillin (Table III). Six of those who had abdominal actinomycosis recovered and two did not. All of those who had pelvic actinomycosis recovered. Of sixteen patients with abdominal actinomycosis who did not receive penicillin, only three appear to have completely recovered.

TABLE III. PENICILLIN THERAPY FOR ABDOMINAL AND PELVIC ACTINOMYCOSIS

TREATMENT	NUMBER OF CASES	RECOVERY		FAILURE	
		NUMBER	PER CENT	NUMBER	PER CENT
Penicillin	11	9	82	2	18
No penicillin	16	3	19	13	81

COMMENT

The results of this study appear to indicate that penicillin is an effective agent in the treatment of actinomycosis, its effectiveness varying somewhat with the location of the lesion. As is true in most infections, actinomycosis varies greatly in different individuals. Apparently in some cases of mild actinomycosis, complete recovery from the infection occurs without treatment.[10] How often spontaneous recovery occurs is difficult to determine, for it is not until the disease has become quite extensive that a clinical and bacteriologic diagnosis can be made. However, when the infection has become well established, spontaneous cures occur only rarely.

Cervicofacial actinomycosis has responded well to several methods of treatment.[11] Prolonged surgical drainage, roentgen therapy, and administration of the iodides and some of the sulfonamide compounds have proved of value in the treatment of actinomycosis when it involves the neck or face. However, penicillin appears to have definitely shortened the duration of the infection and the period of treatment. In cases of cervicofacial actinomycosis of short duration,

the use of penicillin alone has resulted in cures. When the infection has been extensive and of long duration, use of penicillin has been combined with surgical treatment. In most of the cases in which penicillin has been used, the sinuses closed rapidly and the patients recovered completely in a relatively short time. Even the two patients who did not recover completely improved markedly while under treatment. Drainage from the sinuses ceased entirely, and the infection appeared to be cured at the time treatment was discontinued. However, in these two patients evidence of active infection appeared again several months later.

In some cases of cervicofacial actinomycosis death occurs from extension of the infection into the meninges. One of our patients was critically ill, with evidence of meningitis secondary to cervicofacial actinomycosis. Penicillin was administered for six weeks and the patient recovered.

Pulmonary actinomycosis nearly always has been a progressive and fatal disease. The prognosis has been particularly unfavorable when the parenchyma of the lung has been invaded. The usual methods of treatment have been ineffective in most cases. The recovery, after treatment with penicillin, of five of our nine patients who had pulmonary actinomycosis is, therefore, most encouraging. Moreover, two of the patients for whom the results are listed as failures are in good general condition, although they still have evidence of active disease.

Abdominal actinomycosis has always been a serious disease, but good results have been obtained in occasional patients by several different methods of treatment.[12] However, the percentage of patients who recovered has never been very great and the prognosis, therefore, has been poor. Seventy-five per cent of the patients with abdominal actinomycosis who received penicillin recovered. It appears, therefore, that penicillin is a very effective chemotherapeutic agent in the treatment of abdominal actinomycosis.

Actinomycosis involving the pelvic viscera in women may well be merely a localized form of abdominal actinomycosis. However, the prognosis when actinomycosis involves the pelvic organs has been extremely poor, and very few recoveries ever have been reported. The recovery, therefore, after treatment with penicillin, of the three women who had actinomycosis involving the pelvic viscera seems particularly significant.

In the treatment of all types of actinomycosis, a dosage of at least 500,000 units of penicillin daily administered intramuscularly or intravenously for a period of six weeks appears to achieve the best results. Adequate drainage is indicated if abscesses are present.

Because we have been attempting to evaluate the effectiveness of penicillin in the treatment of actinomycosis, other forms of therapy, aside from surgical drainage or excision of diseased tissue, have been avoided so far as possible in the treatment of our patients. It seems possible, therefore, that even better results can be achieved in the future if administration of adequate amounts of penicillin is combined with the use of sulfonamide compounds or of streptomycin.

SUMMARY

Forty-six patients who had actinomycosis were treated with penicillin and have been followed for periods of from one to five years. Of twenty-six patients suffering from cervicofacial actinomycosis, twenty-four had excellent results. These results were obtained after an average period of treatment of less than two months, a period significantly less than the usual length of time required to obtain comparable results when penicillin was not used. Of nine patients who had pulmonary actinomycosis, five recovered. Of eight patients who had abdominal actinomycosis, six recovered. All three patients who had pelvic actinomycosis recovered. All strains of *Actinomyces bovis* cultured from these patients were sensitive to penicillin in vitro. Therefore, penicillin appears to be an effective chemotherapeutic agent in the treatment of actinomycosis and a useful adjunct to other forms of therapy.

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MATERNAL ISOIMMUNIZATION WITHOUT EVIDENCE OF CLINICAL ERYTHROBLASTOSIS FETALIS IN THE NEWBORN

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ONE of the many problems which remain to be solved in the field of Rh-Hr isoimmunization or sensitization is why the occasional infant possessing the antigenic factors against which the mother is isoimmunized is born healthy and shows no subsequent evidence of erythroblastosis. Occasionally, in such cases, the concentration of antibodies of both the early immune and hyperimmune type is surprisingly high. An explanation of this phenomenon may possibly furnish a basis for the development of more effective methods of treating the sensitized mother to lessen or abolish the deleterious effects of the antibodies on the fetus.

Instances of normal Rh-positive infants born to mothers isoimmunized against one or more of the factors which the infant has inherited from the father may be divided into two categories.

1. Cases in which the immunization was discovered at or near term with no clear-cut history of a previously affected infant.

2. The extremely rare case in which there was a history of a previously affected infant.

Several reports have appeared in the literature of clinically normal Rh-positive infants born to Rh-negative mothers whose blood contained Rh antibodies. In the majority of these reports there was no clear-cut history of a previous child suffering from erythroblastosis. Possibly, in these cases, the production of antibodies had been initiated too late in pregnancy to have resulted in significant disease of the fetus or the concentration of antibodies was at no time sufficient to cause fetal damage.

It is probably correct to assume that once isoimmunization against one or more of the Rh-Hr factors has been established, either as a result of pregnancy or transfusion, it is permanent even though the presence of antibodies cannot be detected by present laboratory methods. Experience has shown that in the usual course of events if a mother has been immunized against one or more of the Rh-Hr factors and has borne a child with erythroblastosis, all subsequent infants who inherit one or more of the factors against which the mother is isoimmunized will suffer from erythroblastosis. Furthermore, each succeeding infant is usually affected to a greater degree than its immediate predecessor. Potter,[1] in her monograph, repeatedly substantiates this viewpoint more or less categorically and infers that there are no exceptions to this general rule. She was unable to find any examples in the literature where a normal Rh-positive infant was born to a mother who had previously given birth to an infant suffering from erythroblastosis.

Burnham,[2] Dockeray and Sachs,[3] Goldbloom and Lubinski,[4] and Kariher and Miller[5] all have described cases in which apparently normal Rh-positive

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Aided by a grant from the Banting Research Foundation.
Received for publication, Sept. 4, 1947.
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infants were born to mothers who exhibited at or near term Rh antibodies. In none of the reports was there conclusive evidence of a previous erythroblastotic infant.

Demy[6] published an example of severe erythroblastosis in one of Rh-positive identical twins, with only mild manifestations in the other. Both of the twins survived. The mother was Rh-negative and the father Rh-positive. The mother's blood presumably contained antibodies in high titer. The mother's previous obstetric history was as follows. There were four successive normal pregnancies in 1924, 1927, 1929, and 1932. In 1934 she gave birth to twins, one of whom was stillborn; the other lived four hours and died of hemorrhage. In 1936 she gave birth to a sixteen pound stillborn infant and, although no data were available, it was presumed that this was a fatal case of fetal hydrops.

Although one might deny that the pregnancies terminating in 1934 and 1936 were concerned with Rh isoimmunization, it is difficult to explain the great difference in the degree of involvement of the second set of twins born of the last pregnancy, both of whom survived. The author postulates a greater functional defect in that portion of the placenta serving the twin with the more severe disease. It would seem in this case that other factors, besides the duration of action, concentration, and type of antibodies, influenced the deleterious effect of the antibodies on the fetal tissues. Assuming that the constitutional susceptibility of the identical twins was the same and that the maternal factors were constant for both twins, one has to conclude, as did Demy, that the explanation for the variation was a functional difference in the portion of the placenta serving each twin. It appears that the placenta may play a significant role in the protection of the fetus even if isoimmunization has been established.

Cappell[7] cites an example (Case 10) which is very suggestive of normal Rh-positive children being born after erythroblastosis has been established in earlier offspring. But, as Cappell points out, however suggestive the case history may be, the cause of death in the earlier infants was not fully ascertained, since postmortem examinations were not done. Cappell also states that Dr. Stanbury* and Dr. Chown† both encountered cases in which a healthy unaffected Rh-positive child was born to an Rh-negative mother who had previously delivered a child with severe erythroblastosis. The data on the family studied by Stanbury[8] (Case 3), although very suggestive and probably valid, cannot be accepted as definitely proved since the children presumed to have suffered from erythroblastosis antecedent to the normal infant were born prior to the discovery of the Rh factor and it is not stated whether or not a postmortem was performed.

Diamond[9] has seen nine infants who showed no clinical evidence of erythroblastosis although they were all Rh-positive and were born to women who were isoimmunized to the Rh factor which their babies had.

The two cases which we are reporting are definite exceptions to the usual experience. Fortunately the opportunity presented itself to study these families in some detail. We believe that enough data were obtained to prove that rarely a mother may have an erythroblastotic offspring followed by a healthy infant of

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†Winnipeg.

identical or similar Rh pattern. It is felt that this report is important as it establishes the fact that such cases do occur and thus offers some hope that eventually a method of treatment may be discovered whereby the isommunized mother can be assured of having a normal Rh-positive baby. As far as we can determine, no unequivocal example similar to ours has yet been published.

CASE 1.—Mrs. M. N., Group O, Rh negative, cde/ cde; Mr. G. N., Group O, Rh positive, CDe/CDe (negative to anti-c).

On July 27, 1942, Mrs. N. was delivered of a full-term male infant (P. N.), Group O, Rh positive, CDe/cde. The infant was said to be normal and healthy but was mildly jaundiced on the second day of life. The jaundice disappeared on the same day without treatment and the child has been perfectly well since. No Rh studies were done on the mother at that time.

On May 30, 1945, Mrs. N. delivered a Group O, Rh positive (tested with anti-D only), full-term female infant weighing 6 pounds 12 ounces. Jaundice appeared on the fourth day and became increasingly severe. The infant had peculiar stiffening spells. By the time of admission to the Hospital for Sick Children, on the sixth day of life, the jaundice was extreme. The liver was two fingerbreadths below the right costal margin, but clinically the spleen was not palpated. No nucleated or immature red cells were seen in the blood film. The baby was treated with 100 ml. of normal saline and 60 ml. of blood of unknown Rh type intravenously. Her condition deteriorated rapidly and respirations ceased seventeen hours after admission, seven days after birth.

The mother's serum, tested six days post partum, showed agglutinating or early immune antibodies against Rh-positive cells. Neither titrations nor tests for blocking or hyperimmune type of antibodies were done at this time.

A post-mortem examination was performed. The gross findings were severe jaundice, hepatomegaly, splenomegaly, a marked degree of kernicterus of the basal ganglia, moderate edema of the retroperitoneal tissues, and hemorrhages into the lung parenchyma. Microscopically there was a large amount of extramedullary hematopoiesis in the liver, with a lesser amount in the spleen. Numerous macrophages containing blood pigment were present in the liver and spleen. The findings were in all respects typical of erythroblastosis fetalis (icterus gravis).

On May 7, 1946, Mrs. N. delivered a male full-term infant (F. N.), Group O, Rh positive, CDe/cde. The infant was seen within a few hours of birth by a pediatrician who has a particularly wide experience with erythroblastosis and who, in view of the mother's past history, was expecting the baby to be severely affected. Throughout the infant's normal stay in the maternity hospital there was at no time any clinical evidence of erythroblastosis. The subsequent progress of the child has been normal.

The mother's serum, tested May 16, 1946, nine days post partum, showed blocking or hyperimmune antibodies only, to a dilution of 1:32 against both CDe and cDE cells. Breast milk tested at that time showed the presence of weak antibodies in 1:2 dilution.

Mrs. N.'s serum was again tested January 18, 1947, eight and one-half months post partum, and again only blocking or hyperimmune type of antibodies was present to a dilution of 1:4 against both CDe and cDe cells.

CASE 2.—Mrs. L., Group O, Rh positive, CDe/CDe; Mr. L., Group A, Rh positive, cDE/cde.*

On March 21, 1940, Mrs. L. delivered a male infant (H. L.), Group O, Rh positive, CDe/cDE. The infant survived and was normal. Following delivery the mother required two transfusions. One donor, a brother-in-law, was subsequently found to be Group O, Rh positive, CDe/cDE, and the other was Group O, Rh positive, cDE.

On February 25, 1944, Mrs. L. gave birth to another boy (I. L.), Group A, Rh positive, CDe/cDE. The infant survived and was normal.

*Predicted by Dr. Louis K. Diamond with his anti-d serum and subsequently proved by the last child.

On April 1, 1946, the third boy (R. L.) was born, Group O, Rh positive, CDe/cDE. Jaundice was noted at birth and gradually became more severe. Two days after birth the infant was transferred to The Hospital for Sick Children. Examination on admission revealed a severely jaundiced baby with a palpable liver and spleen. The red blood count was 4,300,000 and the hemoglobin 11.9 grams. Blood films showed many polychromatic red cells and 18 normoblasts per 100 white blood cells. The baby received a total of 465 ml. of Group O, Rh-negative blood* in six transfusions over a seventeen-day period. Plasma and calcium gluconate were also used in the treatment. On discharge at 19 days of age, with a hemoglobin of 13.9 grams, the baby appeared cured. Although the mother states the child was physically slightly retarded at first, in comparison with her other children, subsequent development has been normal.

The third baby was considered to have been a clinical case of erythroblastosis. The antibodies demonstrated in the mother were anti-E, both early immune and hyperimmune, and anti-c, hyperimmune only. Antibody determinations on the mother's serum following the birth of this baby and prior to her last pregnancy were as shown in Table I.

TABLE I. ANTIBODY TITER; MRS. L.'S SERUM USING KNOWN CELLS

	cDe/cDe		cDE/cde		INFANT (R. L.) CDe/cDE		cde/cde	
DATE	SALINE	ALBUMIN	SALINE	ALBUMIN	SALINE	ALBUMIN	SALINE	ALBUMIN
4/7/46	0	0	1:32	1:256	0	1:256	--	--
5/3/46	0	0	1:64	1:128	--	--	0	1:4

In January, 1947, it was learned that Mrs. L. was again pregnant and two samples of blood were obtained prior to her delivery on March 6, 1947. The fourth baby was a full-term male Group O, Rh positive, CDe/cde. Because the child was expected to have a case of erythroblastosis, he was admitted to The Hospital for Sick Children one-half hour after delivery and followed closely throughout his eight-day stay in the hospital. Results of physical examination, red blood count, hemoglobin, and blood film were normal at all times. On admission the hemoglobin was 22 grams. This fell gradually until the day of discharge when it was 16.7 grams. No treatment was given and the subsequent course and development of the child were normal.

TABLE II. ANTIBODY TITER; MRS. L.'S SERUM USING KNOWN CELLS

	cDE/cde		cDe/cde		CDe/CDe		cde/cde	
DATE	SALINE	ALBUMIN	SALINE	ALBUMIN	SALINE	ALBUMIN	SALINE	ALBUMIN
1/17/47	0	0	--	--	0	1:8	0	1:4
2/11/47	--	--	0	1:4	0	1:64	0	1:8
7/18/47	0	0	--	--	±	1:16	0	1:4

Undiluted cord serum and the infant's serum on admission exhibited weak antibodies against Rh-positive CDe/cDE and Rh-negative cells. The infant's cells also gave a 4-plus Coomb's test.

Antibody determinations during and following Mrs. L.'s last pregnancy are shown in Table II. Unfortunately, blood from the mother in the immediate post-partum period was not available.

COMMENT

It is considered that Case 1 is a clear-cut example of a normal Rh-positive child being born to an isoimmunized Rh-negative mother subsequent to the

*Preferably this infant should have received CDe/CDe blood, but at the time of the child's admission facilities were not available for full investigation of the case or Rh subtyping of the donor's blood.

birth of an infant which suffered from erythroblastosis severe enough to cause death. Agglutinating Rh antibodies were demonstrated following the birth of the second child, and postmortem revealed the cause of death of this child to be erythroblastosis. Rh antibodies of the hyperimmune variety were present following the birth of the last child which was Rh positive and clinically normal. Any explanation in the light of our present knowledge must be pure conjecture. If one believes that fetal erythrocytes must cross the placental barrier to induce isoimmunization, a possible explanation, in view of the relatively low titer of antibodies (1:32) present after the birth of the third and normal child, is that during the third pregnancy the placental barrier was intact at all times and that no fetal cells gained entrance to the maternal circulation to further stimulate the production of antibodies to a sufficient level to cause fetal damage. Against this theory is the fact that the titer is not a reliable index of the amount of damage antibodies may cause. Frequently we have encountered persistently low antibody titers, as determined by the ordinary laboratory methods for the agglutinating and blocking or hyperimmune types, in cases where the infant was stillborn or severely involved.

The circumstances in Case 2 are such that a tentative explanation for the normality of the last baby is possible. The isoimmunization of this Rh-positive, CDe/CDe mother, which is of a relatively rare type, is a manifestation of an antibody response to two antigens, E and c. If the relative frequency of the occurrence of antibodies is any criterion, E and c must be considered as weak antigens. The necessary Rh and Hr factors to stimulate the production of the antibodies found in the mother's serum were present in the children of the first three pregnancies, all of which were CDe/cDE, and in the two donors, who were CDe/cDE and cDE, respectively. Presumably three pregnancies and two transfusions were necessary to stimulate antibody production to a sufficient level to result in a moderately severe case of erythroblastosis. It is possible that the isoimmunization was initiated by the two transfusions following the first pregnancy, but the antibody concentration did not reach a sufficient level during the second pregnancy to result in clinical erythroblastosis in that child. During the third pregnancy both anti-E and anti-c were effective against the fetal erythrocytes, the former apparently being the more potent as judged by the titrations. Probably the combined effect of these antibodies, particularly anti-E, was sufficient to result in clinical disease in the infant. In the last pregnancy, resulting in an Rh-positive, CDe/cde boy, only anti-c was effective against the fetal erythrocytes. Although antibodies could be demonstrated in the infant's serum and adsorbed to his erythrocytes, apparently this one effective antibody was not present in sufficient concentrations to have resulted in any appreciable damage to the infant's red blood cells.

SUMMARY

Two cases are reported of maternal Rh-Hr isoimmunization which resulted in infants suffering from erythroblastosis, in each case the mother, in a subsequent pregnancy, delivered a clinically normal baby possessing at least one Rh-Hr factor against which she was isoimmunized.

We wish to acknowledge the kindness of Dr. Louis K. Diamond, Boston, Mass., for providing us with specific Rh antisera and for checking our results. We are also much indebted to Mrs. E. M. Hutchinson for her able technical assistance.

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ANEMIA OF INFECTION

IX. Influence of Adrenalectomy and of Adrenal Cortical Hormone on Hypoferremia and Other Blood Changes Associated With Injection of Turpentine

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OF THE profound metabolic changes accompanying infection, one which seems to be related to the anemia associated with infection is hypoferremia.[1] In view of the role which the adrenal gland has been shown to play in conditions of stress,[2] it seemed desirable to determine whether or not this gland is concerned with this state of hypoferremia. Since a marked fall in plasma iron occurs following injection of either turpentine or staphylococci,[3] the former was adopted as the more easily controllable means for producing the hypoferremia of inflammation. In addition to observations on the plasma iron in normal and adrenalectomized rats following intramuscular injections of turpentine, changes in hemoglobin and in the total and differential white cell counts are recorded.

MATERIALS AND METHODS

One hundred twenty-eight albino rats of both sexes, of Sprague-Dawley strain, each weighing between 100 and 200 grams, were used in this study. The animals were divided into several groups as described under Results. Bilateral adrenalectomy was performed by the dorsal route as described by Griffith and Farris,[4] care being taken to avoid rupture of the capsule and to control bleeding during operation. Following adrenalectomy, these animals were allowed 1 per cent sodium chloride as fluid. The diet otherwise was the same as that of the controls. After seven days, data were secured to establish the range of values for adrenalectomized rats.

Originally it was intended to inject 0.5 ml. of turpentine in normal and in adrenalectomized rats. Unlike the normal controls, the adrenalectomized rats could not withstand this dose of turpentine. But three out of seventeen survived for four hours. By a process of trial and error, a dose of 0.1 ml. of turpentine per 100 grams body weight was selected as one which could be given to adrenalectomized rats as well as to normal unadrenalectomized rats.

Blood for estimation of hemoglobin and total and differential leucocyte counts was obtained from the tail veins of the animals. For plasma iron determinations, groups of three or four were anesthetized with 0.5 ml. of 1 per cent Nembutal per 100 grams body weight in the case of normal intact rats and with 0.4 ml. per 100 grams in the adrenalectomized rats. When fully anesthetized, their abdomens were opened and blood was collected from the abdominal aorta in iron-free syringes and pooled in iron-free centrifuge tubes. Estimation of plasma iron was made from the pooled blood by the method of Barkan and Walker.[5] Hemoglobin was determined photoelectrically with Evelyn's colorimeter.[6] Total and differential white cell counts were done by the usual techniques, cover slip preparations being used for the latter purpose.[6]

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This study was aided by a grant from the United States Public Health Service.
Received for publication, Jan. 16, 1948.
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RESULTS

All data, save for some of the values for plasma iron which consisted of single observations, have been examined by Fisher's "t" test.[7] Mainland[8] has pointed out that this is the only valid method for estimating the significance of means when the number of observations in the series is thirty or less.

Observations in Twenty-One Normal and Fifteen Adrenalectomized Rats.—Data for normal levels of hemoglobin, for total and differential white cell counts, and for plasma iron in normal and adrenalectomized rats are presented in Table I. The values for iron were somewhat lower than had been observed in normal rats in previous experiments in this laboratory.[9] Although monocytes and eosinophiles were encountered in the differential counts, values for these have not been entered in the table as they generally were few in number as compared with other cells and nothing striking was found about them. Table I also gives the comparison of the mean values by the "t" test already referred to. Comparing the values of "t" with those at the usually accepted level of significance (that is, probability of 5 per cent), it is seen that a significant increase

TABLE I. COMPARISON OF BLOOD VALUES IN NORMAL AND ADRENALECTOMIZED RATS

		VALUES IN NORMAL RATS	VALUES IN ADRENALECTOMIZED RATS	DIFFERENCE IN THE RESPECTIVE MEAN VALUES OF THE TWO GROUPS	STANDARD ERROR OF THE DIFFERENCE OF MEAN VALUES	VALUES* OF "t"	REMARKS
Plasma iron (μg per 100 ml.)	Number of observations	4	5				
	Mean	202	166	-36	±36	1.00	Not significant
	Range	121-262	133-252				
	Standard deviation	±59	±49				
Hemoglobin (Gm. per 100 ml.)	Number of observations	14	13				
	Mean	13.91	14.94	+1.03	±0.39	2.64	Significant
	Range	12.69-15.15	12.98-16.80				
	Standard deviation	±0.77	±1.24				
Total white cells (per c.mm.)	Number of observations	16	14				
	Mean	18,175	20,289	+2,114	±2,070	1.02	Not significant
	Range	10,950-31,000	12,600-29,600				
	Standard deviation	±6,100	±5,103				
Neutrophiles (per c.mm.)	Number of observation	16	14				
	Mean	3,562	7,253	+3,691	±969.2	3.81	Significant
	Range	1,403-6,442	2,520-13,170				
	Standard deviation	±1,743	±3,463				
Lymphocytes (per c.mm.)	Number of observations	16	14				
	Mean	14,021	12,266	-1,755	±1,285	1.37	Not significant
	Range	8,586-23,481	7,923-14,800				
	Standard deviation	±4,450	±2,123				

*Calculated by dividing the difference in the mean values by their standard errors.

in hemoglobin and in neutrophiles was observed following adrenalectomy but no significant changes could be demonstrated in total white cell count, lymphocytes, or plasma iron.

Observations on the Effects of Injection of Turpentine in Normal and Adrenalectomized Rats.—

1. In a series of twenty-one rats, irrespective of individual weights, 0.5 ml. of turpentine was injected in the leg. Blood studies were made at intervals of four, eight, twelve, twenty-four, forty-eight, and ninety-six hours after injection. Data for plasma iron and hemoglobin obtained from these animals, collected in Table II, show a rise in hemoglobin level reaching its maximum at twelve

TABLE II. FLUCTUATIONS IN LEVEL OF PLASMA IRON AND HEMOGLOBIN IN RATS INJECTED WITH 0.5 ML. OF TURPENTINE, AND SIGNIFICANCE OF HEMOGLOBIN VALUES AS COMPARED WITH THOSE FOR NORMAL UNTRAUMATIZED RATS

HOURS AFTER INJECTION	PLASMA IRON (μG/100 ML.)	HEMOGLOBIN		
		GM. PER 100 ML.	VALUES OF "T"	REMARKS
4	175.54	16.29	3.45	s.
8	117.90	16.94	6.06	
12	73.70	17.69	7.56	
24	47.02	15.74	3.81	
48	152.96	13.95	0.09	n.s.
96	159.89	15.20*	2.32	s.

*Mean of two observations only.
s, Significant; n.s., not significant.

hours and a fall in plasma iron reaching its lowest level at twenty-four hours. A sudden drop of total leucocytes and lymphocytes, accompanied by a slower drop in granulocytes, is evident from Fig. 1, where the values for these have been charted. The significance of these values as compared with data for normal untraumatized controls is indicated in Table III.

TABLE III. FLUCTUATION IN VALUES FOR WHITE CELLS IN RATS RECEIVING 0.5 ML. OF TURPENTINE AND THEIR SIGNIFICANCE AS COMPARED WITH VALUES FOR NORMAL UNTRAUMATIZED RATS

HOURS AFTER INJECTION	TOTAL WHITE CELLS			LYMPHOCYTES			NEUTROPHILES		
	NUMBER PER C.MM.	VALUE OF "T"	REMARKS	NUMBER PER C.MM.	VALUE OF "T"	REMARKS	NUMBER PER C.MM.	VALUE OF "T"	REMARKS
4	11,583	1.82	n.s.	5,987	3.05	s.	5,497	1.80	n.s.
8	6,667	3.18	s.	4,546	3.98		1,889	1.71	
12	3,100	4.18		1,985	4.58		961	2.67	s.
24	7,567	2.92		5,721	3.12		1,614	2.01	n.s.
48	12,500	1.55	n.s.	9,783	1.58	n.s.	2,240	1.35	
96	15,700*	0.66	n.s.	10,889	0.96	n.s.	4,217	0.54	

*Mean of two values only.
s, Significant; n.s., not significant.

2. In a series of twenty-four normal intact rats turpentine was injected in a dose of 0.1 ml. per 100 grams. Data from these rats are summarized in Tables IV and V. As can be seen from Fig. 2, with this dose normal intact rats reacted in essentially the same manner as those receiving 0.5 ml. of turpentine.

The leucocyte curves exhibited more or less the same features as those of rats receiving 0.5 ml. of turpentine; the drop in values was, however, less

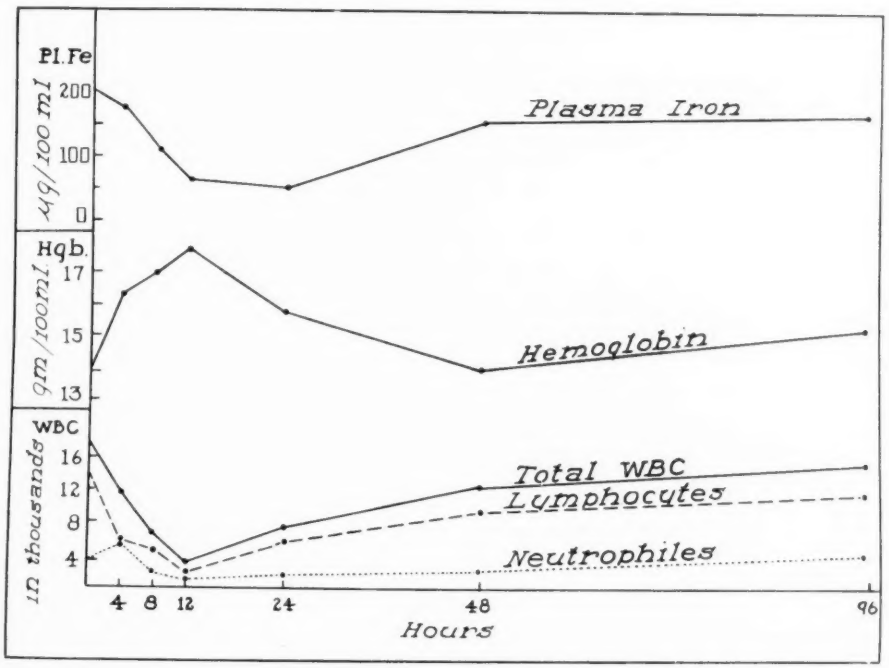

Fig. 1.—Changes in plasma iron, hemoglobin, and white cells in normal intact rats receiving 0.5 ml. of turpentine injected at 0 hour.

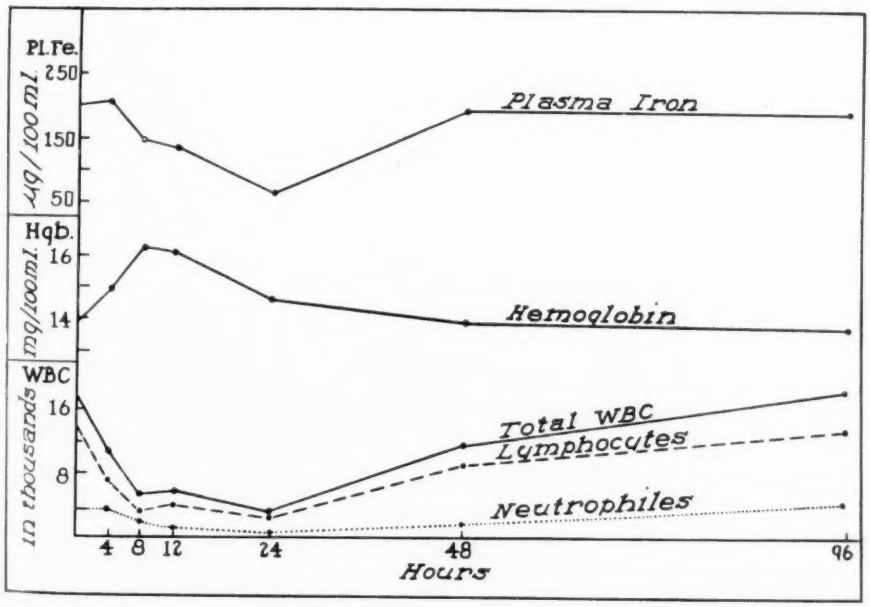

Fig. 2.—Changes in plasma iron, hemoglobin, and white cells in normal intact rats receiving 0.1 ml. of turpentine per 100 grams injected at 0 hour.

TABLE IV. FLUCTUATIONS IN LEVEL OF PLASMA IRON AND HEMOGLOBIN IN RATS INJECTED WITH 0.1 ML. OF TURPENTINE, AND SIGNIFICANCE OF HEMOGLOBIN VALUES AS COMPARED WITH THOSE FOR NORMAL UNTRAUMATIZED RATS

HOURS AFTER INJECTION	PLASMA IRON (μG/100 ML.)	HEMOGLOBIN		
		GM. PER 100 ML.	VALUES OF "T"	REMARKS
4	208.73	14.92	1.70	n.s.
8	148.51	16.27	5.13	s.
12	137.23	16.15	4.76	
24	66.00	14.67	1.61	
48	195.30	13.97	0.13	n.s.
96	188.15	13.83	0.17	

s. Significant; n.s., not significant.

precipitous, the lowest value being delayed until twenty-four hours after injection. The significant drop in leucocytes occurred at practically the same period, as will be seen from Table V.

TABLE V. FLUCTUATION IN VALUES FOR WHITE CELLS IN RATS RECEIVING 0.1 ML. OF TURPENTINE AND THEIR SIGNIFICANCE AS COMPARED WITH VALUES FOR NORMAL UNTRAUMATIZED RATS

HOURS AFTER INJECTION	TOTAL WHITE CELLS			LYMPHOCYTES			NEUTROPHILES		
	NUMBER PER C.MM.	VALUE OF "T"	REMARKS	NUMBER PER C.MM.	VALUE OF "T"	REMARKS	NUMBER PER C.MM.	VALUE OF "T"	REMARKS
4	10,967	1.99	n.s.	7,095	2.61	s.	3,688	0.13	n.s.
8	5,517	3.50	s.	3,277	4.08		2,182	1.41	
12	5,750	3.43		4,119	3.74		1,532	2.08	s.
24	3,583	4.04		2,713	4.29		794	2.85	
48	11,833	1.72	n.s.	9,425	0.88	n.s.	2,019	1.58	n.s.
96	18,700	0.13		13,735	0.10		4,577	0.97	

s. Significant; n.s., not significant.

3. In twenty-four adrenalectomized rats 0.1 ml. of turpentine per 100 grams weight was injected and data collected as before. As is evident from Fig. 3, although there was the same tendency toward a fall in plasma iron and a rise in hemoglobin curve within the first twelve hours as in traumatized unadrenalectomized rats, the subsequent changes differed markedly. Thus Table VI brings out the fact that from twenty-four hours and on after injection, the hemoglobin in these animals seemed to be stabilized at a significantly lower level than before injection and, concurrently, there was a definite lag in the rise of the plasma iron curve.

TABLE VI. FLUCTUATIONS IN LEVEL OF PLASMA IRON AND HEMOGLOBIN IN ADRENALECTOMIZED RATS RECEIVING 0.1 ML. OF TURPENTINE, AND SIGNIFICANCE OF HEMOGLOBIN VALUES AS COMPARED WITH THOSE FOR UNTRAUMATIZED ADRENALECTOMIZED RATS

HOURS AFTER INJECTION	PLASMA IRON (μG/100 ML.)	HEMOGLOBIN		
		GM. PER 100 ML.	VALUES OF "T"	REMARKS
4	150.65	14.86	0.10	n.s.
8	117.22	16.17	1.32	
12	52.76	15.52	0.73	
24	69.08	12.63	2.88	s.
48	88.58	13.22	2.24	
96	96.02	12.61	2.87	

s. Significant; n.s., not significant.

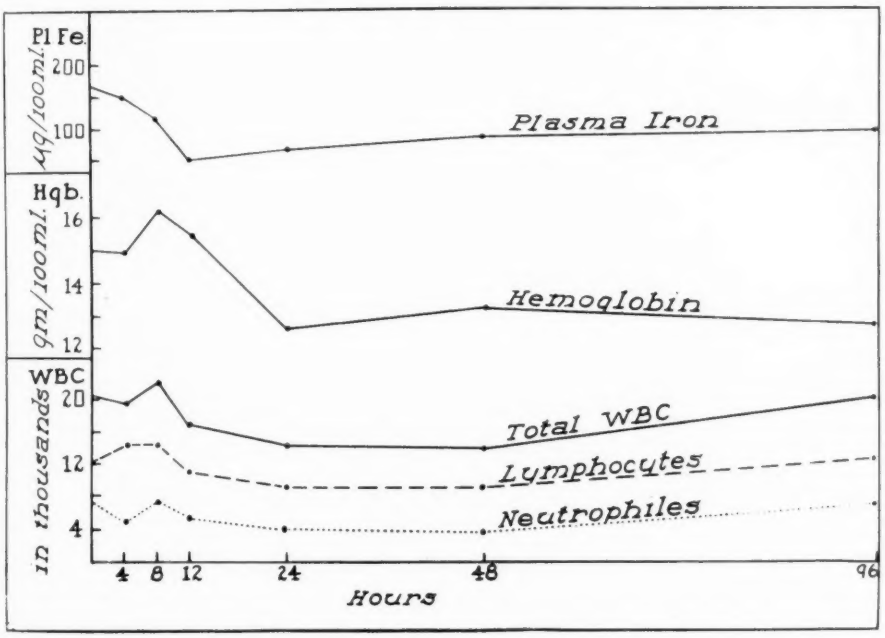

Fig. 3.—Changes in plasma iron, hemoglobin, and white cells in adrenalectomized rats receiving 0.1 ml. of turpentine per 100 grams injected at 0 hour.

With regard to the leucocyte picture, there was a marked departure from the pattern observed in all the previous groups in that no significant alterations took place (Fig. 3, Table VII).

TABLE VII. FLUCTUATIONS IN VALUES FOR WHITE CELLS IN ADRENALECTOMIZED RATS RECEIVING 0.1 ML. OF TURPENTINE AND THEIR SIGNIFICANCE AS COMPARED WITH THOSE FOR UNTRAUMATIZED ADRENALECTOMIZED RATS

HOURS AFTER INJECTION	TOTAL WHITE CELLS			LYMPHOCYTES			NEUTROPHILES		
	NUMBER PER C.MM.	VALUE OF "T"	REMARKS	NUMBER PER C.MM.	VALUE OF "T"	REMARKS	NUMBER PER C.MM.	VALUE OF "T"	REMARKS
4	19,267	0.26	n.s.	14,234	1.19	n.s.	4,874	1.17	n.s.
8	22,167	0.61		14,384	1.64		7,532	0.12	
12	16,817	1.14		11,078	0.76		5,365	0.82	
24	14,284	1.96		9,750	1.74		4,134	1.43	
48	13,933	2.08		9,750	1.99		3,828	1.65	
96	20,300	0.00		12,720	0.30		6.940	0.14	

n.s., Not significant.

Observations on the Effects of Cortical Hormone on Normal Rats.—In a series of twenty intact normal rats, adrenal cortical extract* was injected intramuscularly in doses of 1.0 ml. per rat without regard to individual weights. This dose is double that used by Dougherty and White[10-12] in their experiments and was chosen advisedly in order to emphasize the effects, if any, of cortical hormone on the level of plasma iron in rats. As is evident from Fig. 4, there

*Adrenal cortical extract was furnished by the Upjohn Company, Kalamazoo, Mich.

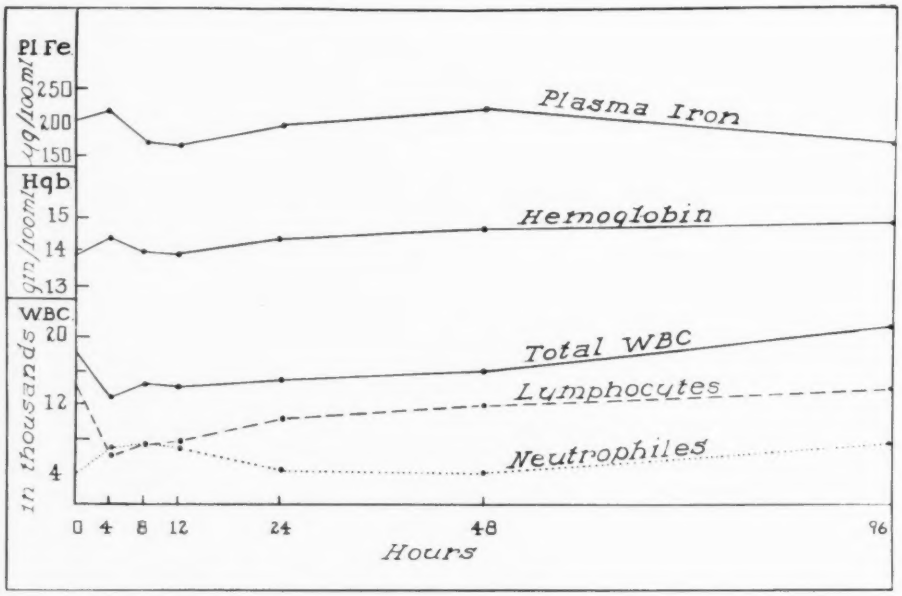

Fig. 4.—Changes in plasma iron, hemoglobin, and white cells in normal intact rats receiving 1.0 ml. of cortical hormone injected at 0 hour.

TABLE VIII. FLUCTUATIONS IN LEVEL OF PLASMA IRON AND HEMOGLOBIN IN RATS RECEIVING 1.0 ML. OF CORTICAL HORMONE, AND SIGNIFICANCE OF HEMOGLOBIN VALUES AS COMPARED WITH THOSE FOR NORMAL RATS NOT RECEIVING SUCH INJECTIONS

HOURS AFTER INJECTION	PLASMA IRON (μG/100 ML.)	HEMOGLOBIN		
		GM. PER 100 ML.	VALUES OF "T"	REMARKS
4	217.34	14.42	1.10	n.s.
8	172.71	14.01	0.21	
12	166.81	13.96	0.10	
24	196.81	14.39*	0.86	
48	222.39	14.68	1.49	
96	172.71	14.86	2.02	

*Mean of two observations only.
n.s., Not significant.

TABLE IX. FLUCTUATIONS IN NUMBER OF WHITE CELLS IN RATS RECEIVING 1.0 ML. OF CORTICAL HORMONE AND THEIR SIGNIFICANCE AS COMPARED WITH VALUES FOR NORMAL RATS NOT RECEIVING SUCH INJECTIONS

HOURS AFTER INJECTION	TOTAL WHITE CELLS			LYMPHOCYTES			NEUTROPHILES		
	NUMBER PER C.MM.	VALUE OF "T"	REMARKS	NUMBER PER C.MM.	VALUE OF "T"	REMARKS	NUMBER PER C.MM.	VALUE OF "T"	REMARKS
4	12,733	1.50	n.s.	5,657	3.11	s.	6,777	3.14	s.
8	14,300	1.08		6,992	2.67		7,123	3.08	
12	14,167	1.10		7,106	2.62		6,902	2.97	
24	14,633	0.96		10,110	1.47	n.s.	4,206	0.69	n.s.
48	15,883	0.63		11,746	0.86		3,793	0.23	
96	21,033	0.77		13,650	0.13		7,274	3.59	s.

s, Significant; n.s., not significant.

did not seem to be much variation in the levels of plasma iron or hemoglobin. This impression is confirmed also by examination of Table VIII where the values for these rats have been summarized.

The behavior of the leucocytes was very striking, however, as is evident from Fig. 4. Within four hours after injection there was a fall in the number of lymphocytes, with a corresponding increase in the number of neutrophiles as well as some variation in the total white cells. The significance of these variations is given in Table IX.

DISCUSSION

Neither adrenalectomy nor injection of cortical hormone produced any significant change in the value of plasma iron in normal rats. On the other hand, both in normal intact and in adrenalectomized rats the injection of turpentine was immediately followed by a significant drop in the level of plasma iron. Thus, hypoferremia following turpentine injection is independent of the presence or absence of the adrenal glands in rats. However the hypoferremia seemed to persist longer in adrenalectomized than in nonadrenalectomized rats. Further, in adrenalectomized rats, this prolonged hypoferremia was associated with a significantly lower level of hemoglobin. It appears, therefore, that the adrenal gland may be related in some way to the quick recovery from hypoferremia which occurs in intact rats injected with turpentine and to the maintenance of the normal hemoglobin level.

Adrenalectomy was followed by a significant rise in hemoglobin values in rats. This was probably due to hemoconcentration which is known to occur in most animals after adrenalectomy. No significant change in hemoglobin was noticed in normal rats on injection of cortical hormone. The immediate effect of turpentine injection on the hemoglobin values of both normal and adrenalectomized rats was a sudden rise. This was more marked in the normal than in the adrenalectomized rats.

The number of total leucocytes was not significantly altered either by adrenalectomy or by injection of cortical hormone in normal rats. Our findings, here, are not in agreement with those of Dougherty and White[10-12] in whose hands there was an increase following adrenalectomy and a decrease on injection of cortical extract. Corey and Britton[13] observed a fall in leucocytes in cats following adrenalectomy. The effect of turpentine injection on the total leucocytes of normal intact rats and adrenalectomized rats differed markedly. In the normal nonadrenalectomized rats there was a sharp and significant drop in leucocytes; in the adrenalectomized rats, on the contrary, no such significant fall was noticed. It seems, therefore, that the adrenal gland may be in some way connected with the peripheral leucopenia that occurs in normal rats following turpentine injection.

Both adrenalectomy and the injection of cortical extract in normal rats caused a significant rise in the number of neutrophiles. These results are difficult to interpret. The turpentine injection caused a significant drop of neutrophiles in normal rats, but no such effect was observable in adrenalectomized rats.

In our hands, adrenalectomy did not significantly alter the number of lymphocytes. The injection of cortical hormone in normal rats, however, produced a significant drop in the number of lymphocytes and this finding is in accord with that of Dougherty and White.[10-12] The effect of turpentine injection on the lymphocytes was the same as on neutrophiles inasmuch as it produced a significant drop in the normal intact animals but none at all in adrenalectomized rats.

From the foregoing, it is clear that although the adrenal gland does not affect hypoferremia as such, its presence may possibly be necessary for the quick recovery from hypoferremia as well as for the delay in the onset of anemia such as occurs from turpentine injection. The adrenal gland may also be involved in the leucopenia which follows the injection of turpentine.

SUMMARY AND CONCLUSION

A series of one hundred twenty-eight albino rats was used in this study. Of these, twenty-one served as normal controls, forty-five received injections of turpentine in two separate groups in doses of 0.5 and 0.1 ml., respectively, while twenty were injected with adrenal cortical extract. Of the remaining forty-two, which were all adrenalectomized, fifteen served as controls, twenty-four received 0.1 ml. and three 0.5 ml. of turpentine. Plasma iron, hemoglobin, total and differential white cell counts were determined in these animals. Information regarding the original data has been condensed into a number of tables and the significance tested.

The following conclusions have been reached:

1. The adrenal gland has no effect on the normal level of plasma iron in rats, nor does it affect the hypoferremia which results in these animals from turpentine injection.

2. The adrenal gland may, however, be related in some way to the quick recovery from hypoferremia induced by the injection of turpentine.

3. The adrenal gland does not seem to have any influence on the maintenance of the normal hemoglobin level in rats, though under conditions of stress, such as turpentine injection, it may play some role in preventing a drop in the level of hemoglobin that might otherwise occur.

4. The adrenal gland is intimately concerned with the peripheral leucopenia that follows turpentine injection in normal rats. This leucopenia involved both lymphocytes and granulocytes and was not observed in adrenalectomized rats.

The guidance of Dr. George Sayers in performing the adrenalectomies is gratefully acknowledged.

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IDIOPATHIC (FAMILIAL) HYPOPROTHROMBINEMIA

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RHOADS and Fitz-Hugh in 1941[1] first recorded an instance of idiopathic hypoprothrombinemia as the basis for an hemorrhagic diathesis. Since then eight* additional cases have been reported.[2, 6-11] Hypoprothrombinemia has been noted in several individuals otherwise normal or in persons who have had only one significant bleeding episode.[3a, 5, 6, 10] Familial hypoprothrombinemia has been demonstrated in at least four instances.[2, 5, 6, 10]

Most of the patients thus far reported were not followed in detail over a long period of years. Some reports lack sufficient data to establish beyond doubt that the prolonged prothrombin time is due to prothrombin deficiency. Table I summarizes the cases of idiopathic hypoprothrombinemia from the literature.

The patient who formed the basis for the present study was seen repeatedly since December, 1937, on various services at the University Hospital. Besides numerous outpatient visits there were forty-seven hospital admissions. During this period a considerable mass of clinical and laboratory data was collected. The purpose of the present report is to summarize these observations, to describe recent and more detailed studies of the patient's abnormality, and to compare the information gained with that contained in the literature.

CASE REPORT

J. L., a 20-year-old female domestic, first visited the University Hospital Out-Patient Department in December, 1937, for study and treatment of an eight-year bleeding tendency.

Past Health.—Birth and neonatal periods, as well as subsequent development, were entirely normal. The patient had the usual childhood diseases, but except for the hemorrhagic tendency her past health was good.

Family History.—The parents were of Swedish extraction. Their families had no blood relatives in common. The mother's paternal grandmother was said to be a bleeder. The mother had always bruised easily but had never had abnormal bleeding. No one else in the patient's ancestry was known to have a hemorrhagic tendency. The patient's siblings were normal except that the youngest, now 13 years old, had severe melena at 2 weeks of age. One brother (R.) had severe rheumatic fever. Detailed studies of the immediate family will be given.

Present Illness.—Nothing abnormal was observed until the child was 2 years of age when she developed a swollen, discolored, painful left knee apparently without trauma. From that time on the course was characterized by recurrent episodes of various painful joints, epistaxis (up to the menarche), ecchymotic areas and deep, painful hematomas, both apparently spontaneous and obviously posttraumatic, persistent bleeding from lacerations, a few showers of petechiae especially over the buttocks, abdominal pain, and menorrhagia and metrorrhagia from the onset of menses until a recent hysterectomy. It would be difficult to say that the patient had true hemarthrosis. The joints usually were not swollen, blood was not aspirated, and no limitation of motion of any joint resulted. There never was significant hematuria.

From the Department of Medicine, University of Minnesota Hospital.
Received for publication, April 5, 1948.

*Beard's case cited by Quick[11a] is not included since recent study[6a] showed no hypoprothrombinemia.

TABLE I. CASES REPORTED AS IDIOPATHIC HYPROTHOMBINEMIA

PATIENT	REPORTED BY	YEAR	HISTORY	FAMILY HISTORY	HEMATOLOGIC STUDIES	PROTHROMBIN TIME AND THERAPEUTIC RESPONSE
1	Rhoads and Fitz-Hugh[1]	1941	♂, 18 yr. Onset at 9 months with probable hemarthrosis right knee; course—repeated tooth socket and gum hemorrhages, prolonged bleeding from cuts and lacerations, hematomas, hemarthrosis and hematuria; 26 hospital admissions from 1929 to 1940; death due to cerebral hemorrhage	Negative for any abnormal hemorrhagic tendency	BT (Duke), 2-25 min., more often normal; CT (venous) 8-360 min., almost always prolonged; CR variable; PC normal; TT negative once, positive once (25 petechiae); F normal quantity, but qualitative defect believed present; anticoagulants believed ruled out	Quick, 70-120 sec.; controls, 20-24 sec.; refractory to vitamin K; blood apparently had hemostatic effect
2	Giordano[2]	1943	♂, 22 yr. Onset at 5 years with frequent, spontaneous epistaxis; course—recurrent epistaxis, tooth socket and gum hemorrhages, hemarthrosis; at times patient noted skin petechiae and moderate icterus	Both parents and 2 siblings hypoprothrombinemic; maternal aunt and sister known bleeders; parents and 2 siblings had positive TT; father's BT 11½ min.; all had decreased serum proteins (5.34-5.99 gm. %)	BT 7.5 and 4 min.; CT, CR, and PC normal; TT positive; F 0.48 Gm. %; anticoagulant study not specifically mentioned (probably not present; addition of patient's plasma to control plasma did not prolong PT beyond that expected from dilution)	Smith, 9% of normal; refractory to vitamin K; blood had apparent hemostatic effect; plasma increased prothrombin in vivo
3	Plum[3a]	1943	♀, 20 yr. Apparently normal individual; hypoprothrombinemia detected incidentally; however, "a few times had strong and long-lasting nosebleed"	No information given (Heinild[3b, c] reports 1 brother had severe epistaxis, 2 nephews have fibrinopenia and hypoprothrombinemia)	CT, spontaneous coagulation of recalcified blood (Larsen and Plum), 202 sec. (normal 120-150 sec.); CR and PC normal; TT negative; F .204 Gm. % (Gram)	Larsen and Plum, 30.5 sec.; control, 18 sec.; refractory to vitamin K
4	Plum[3a]	1943	♂, 18 yr. Normal individual; hypoprothrombinemia detected incidentally	No information given	CT, spontaneous coagulation of recalcinated blood (Larsen and Plum), 257 sec. (normal 120-150 sec.); PC normal; TT negative	Larsen and Plum, 28 sec.; control, 18 sec.; refractory to vitamin K

BT, Bleeding time; CT, clotting time; CR, clot retraction; PC, pla telet count; TT, tourniquet test for capillary fragility; F. fibrinogen; PT, prothrombin time.

(Cont'd on next page)

TABLE I—CONT'D

PATIENT	REPORTED BY	YEAR	HISTORY	FAMILY HISTORY	HEMATOLOGIC STUDIES	PROTHROMBIN TIME AND THERAPEUTIC RESPONSE
5	Quick[4-6]	1943	♂, 22 yr. Normal individual; hypoprothrombinemia detected incidentally	Mother and sister hypoprothrombinemic (a little lower than 50%); sister has had several episodes of uterine bleeding	BT normal; CT 5-6 min.; CR and PC normal; F quantitatively normal; anticoagulants believed ruled out	Quick, 45% of normal; deficiency of Quick's component B; refractory to vitamin K
6	Murphy and Clark[7]	1944	♂, 18 yr. Onset at 4 years with profuse, persistent epistaxis following traumatic fracture of nose; course—recurrent epistaxis; gum hemorrhages; one episode of hematuria	Sister died at 5 years following severe, recurrent epistaxis; father slightly hypoprothrombinemic (PT, Quick, 19 sec., control, 16 sec.). (authors believed this was probably of no significance)	BT (Duke) 1½-15 min., more frequently normal; CT, CR, and PC normal; TT negative; microscopic examination of nail bed capillaries revealed "definitely abnormal and bizarre forms"; F 0.331 Gm. %, ". . . definitely suggestive evidence of some type of qualitative defect"; anticoagulants believed ruled out	Quick, 60-100 sec.; control, 16 sec; refractory to vitamin K; blood found to have hemostatic effect
7	de Marval and Bomchil[8]	1944	♀, 14 year. Onset of epistaxis at 8 years; course—recurrent epistaxis, then gum hemorrhages at 14 years	Parents distantly consanguineous; no bleeders known	BT normal; CT 12-40 min.; CR and PC normal; TT slightly positive at times, negative at times; F 0.56 Gm. %	Quick, 25-53% of normal; refractory to vitamin K
8	de Marval[9]	1945	♀, 23 yr. Onset at 3 years with profuse bleeding from dog bite; course—recurrent tooth socket and gum hemorrhages, hemarthrosis, menorrhagia; one normal pregnancy (apparently no abnormal hemorrhages at parturition)	Parents were cousins; father died at 39 of an hemorrhagic disease; mother normal, but had many spontaneous abortions; four of 9 brothers died in infancy, cause unknown	BT normal; CT 10-12 min. (author indicates that these levels are prolonged); CR and PC normal; TT negative	Quick, 20-25% of normal; refractory to vitamin K

6	Hauser[10]	1945	♂, 3 yr. Onset at 3 months with prolonged bleeding from a needle puncture and bleeding from the mouth; course—recurrent epistaxis, easy bruising, severe hemorrhage during maxillary cyst operation at 2 years; hospitalized for severe epistaxis at 3 years	Parents distantly consanguineous; father slightly hypoprothrombinemic (index 77%); brother (Patient 10) hypoprothrombinemic	BT 2-2.5 min.; CT (Bürker) 3 min. 7 sec.-11 min. 20 sec., usually prolonged; CR and PC normal; TT negative; F 0.34 and 0.21 Gm. %; anticoagulants believed ruled out	Index, 21-80%; refractory to vitamin K
10	Hauser[10]	1945	♂, Brother of Patient 9; normal but had one episode of severe epistaxis	See Patient 9	BT normal; CT (Bürker) 5 min. 45 sec.-14 min.; CR and PC normal; TT negative; F quantitatively normal	Index, 53%; refractory to vitamin K
11	Lewis and Bennett[11, 12]	1947	♀, 29 yr. Abrupt onset at 29 years; nasal, gum, subcutaneous, intramuscular, uterine, and urinary bleeding of 5 days' duration	Maternal aunt bruised easily	BT 45 sec.; CT 60 min.; CR slow and incomplete; TT negative; F normal; anticoagulants believed ruled out; antiprotease factor normal	Quick, 5-10 min.; control, 18 sec.; deficiency of Quick's component B; PT normal after 3 days of massive doses vitamin K, blood, and plasma
12	Quick[6]	1947	♂, 1 yr. Onset shortly after birth with tongue and bowel hemorrhages; prolonged bleeding from lacerations	Brother (Patient 13) hypoprothrombinemic; grandparents on both sides, parents, and one brother normal (sister not examined)	BT normal; CT 12-12.5 min.; CR and PC normal; F quantitatively normal; anticoagulants believed ruled out	Quick, 19 sec.; controls, 11-12.5 sec; component assay: lack of neither "prothrombin (component B) [nor] the labile factor but . . . a new coagulation factor"; refractory to small doses of vitamin K
13	Quick[6]	1947	♂, 5½ yr. Brother of Patient 12; onset at 1 week with 3 months' bleeding after circumcision; course—easy bruising, tooth socket hemorrhage at 5 years	See Patient 12	BT normal; CT 12-13 min.; CR and PC normal; F quantitatively normal; anticoagulants believed ruled out	Same as Patient 12

The patient was first admitted to the University Hospital in March, 1938, for various studies. From then until the onset of menstruation in January, 1941, there were three admissions for epistaxis or prolonged bleeding from a laceration. From the menarche until hysterectomy in September, 1945, there were thirty-seven admissions concerned primarily with the uterine bleeding. Moreover, the other symptoms mentioned recurred over this period.

After the hysterectomy there were four hospitalizations here and one elsewhere. Two were for exacerbations of ecchymoses, painful deep hematomas, and various joint aches, while three admissions were for probable intra-abdominal hemorrhage. Pelvic hematomas were present on two occasions. The last admission was in March, 1947.

Various studies were made and a variety of medications was tried during these hospitalizations. However, no cause for the bleeding other than hypoprothrombinemia was demonstrated. Although vitamin K preparations, vitamin C, various hormones, and other drugs were used repeatedly, the only effective therapeutic measure was transfusion of blood or plasma.

The patient was receiving almost weekly infusions of plasma at the time of writing. This minimized but did not eliminate entirely the tendency to ecchymoses and deep hematomas. The joint aches, however, seemed to be well controlled. The last five hospitalizations occurred during periods when the plasma injections were not given with regularity.

Physical Examination.—The patient was a well-developed, robust appearing young woman. Examinations usually were within normal limits except for the ecchymotic areas and the deep hematomas from time to time. The hematomas usually were in the extremities, especially the lower extremities. No cardiac abnormalities were noted. The liver and spleen were not enlarged. No spider nevi were seen. A pelvic hematoma large enough to push the cervix to the introitus and palpable three fingers above the symphysis pubis was present, as mentioned. In recent years no significant abnormalities were noted about the joints even when there was aching; none had limitation of motion. Neurological examination always was normal.

Laboratory Findings.—Approximately 900 laboratory results were recorded. They can be summarized as follows.

The hemoglobin and erythrocyte values were normal except during the times of severe hemorrhages. The lowest levels resulting from epistaxis and laceration were 8.8 Gm. hemoglobin and 3,350,000 red blood cells, while during the period of uterine bleedings the values fell as low as 4.4 Gm. hemoglobin and 1,460,000 red blood cells. White blood cells, differential and platelet estimations were normal. Results of bone marrow examination Mar. 27, 1940, were not remarkable. Urinalyses showed no abnormalities if catheterized specimens were examined. Neither gross nor microscopic hematuria ever was proved. Blood calcium and vitamin C levels were normal. Plasma proteins including fibrinogen repeatedly were found within normal limits. Serologic tests for syphilis were negative. The patient's blood type was A. Liver function tests consistently showed only one aberration, namely a positive cephalin-cholesterol flocculation test, as detailed in Table II. Serum bilirubin levels were normal except for one elevation noted in 1941—an icterus index of 35 which was checked three

TABLE II. CEPHALIN-CHOLESTEROL FLOCCULATION

DATE	24 HR.	48 HR.
5/17/44	3+	4+
8/23/44	3+	3+
8/29/44	4+	4+
12/11/44	1+	2+
12/13/44	2+	3+
3/ 4/46	1+	2+
12/30/46	1+	1+
3/22/47	2+	2+

days later and found to be 4. There were no symptoms of hepatitis noted at that time or recalled by the patient. She had received 1,000 c.c. of blood the preceding two days but no reaction was noted. Thymol turbidity, bromsulfalein, urine and fecal urobilinogen, urine coproporphyrin, cholesterol, cholesterol esters, alkaline phosphatase, hippuric acid excretion, galactose tolerance, and stercobilin clearance tests were all within normal limits.

The usual tests of hemostasis gave variable results. Sixty-three per cent of forty-nine clotting time determinations were prolonged. The clotting time was prolonged to 55 minutes twice, once in 1938 and again in 1947. Approximately 40 per cent of thirteen retraction studies were abnormal. Bleeding times were prolonged in 43 per cent of forty-six tests. Bleeding time recently was over 37 minutes, but usually when prolonged it was only mildly so. The cuff test was usually negative; only two tests out of thirteen were positive.

Prothrombin times were consistently elevated and in the same range over this period as illustrated by Table III.

TABLE III

DATE*	PROTHROMBIN TIMES (QUICK) (SEC.)		CONCENTRATION OF PROTHROMBIN IN PLASMA (%)
	CONTROL	J. L.	
5/ 9/38	12	81	< 5
5/17/44	11	67	< 5
7/13/44	11.5	51.5	<10
8/23/44	12	50	<10
9/ 8/44	12	74	< 5
9/20/44	12	47	<10
10/13/44	12.5	57	5
5/26/47	12	56.5	5
6/25/47	12.5	47.5	<10
7/ 3/47	12	52.5	<10

*Selected from approximately 140 determinations on the basis of the control values which fall within Quick's 11 to 12.5 second range. This facilitates expression in per cent by Quick's chart.[14, b]

Special Studies.—As Quick[15] has emphasized, prothrombin is estimated by a measure of its activity. The estimation is based upon certain assumptions. To prove that a delay in prothrombin time is due to prothrombin deficiency, other possible causes must be excluded. Several studies were made in the present case with this objective in mind.

TABLE IV. CLOTTING TIME OF RECALCIFIED PLASMA[14, c]

	LOW SPEED CENTRIFUGATION (SEC.)	HIGH SPEED CENTRIFUGATION (SEC.)	VENOUS CLOTTING TIME (MIN.)	PROTHROMBIN TIME
Normal plasma	113	141		
Hemophiliac plasma	281	433	55	17.5/18 (control)
J. L.'s plasma	742	760	51	39.7/14.4 (control)

1. *Effect of Platelets:* In Quick's test the thromboplastic factor is assumed to be eliminated by the addition of an excess of thromboplastin. Nevertheless it was thought of interest to examine the effect of platelets in the present case. This was done by comparing the recalcified clotting times of low and high speed centrifugalized plasma as outlined by Quick.[14, c] The test was carried out,* with the results outlined in Table IV. These results reveal a behavior quite unlike that in hemophilia.

*By Miss Elaine Daughenbaugh, Instructor, School of Medical Technology, University of Minnesota.

2. *Effect of Purified Prothrombin* in vitro: This study was made in order to exclude the possibility of an interference with the conversion of prothrombin. This might be caused by a factor such as excessive trypsin inhibitor[16] or, as recently suggested,[17] by a lack of plasma accelerator factor (Ac-globulin). Purified prothrombin* was prepared by the method of Seegers and associates.[18] A comparison was made of the effect of progressive concentrations of this material on the prothrombin times of old plasma, dicumarolized plasma, and plasma from our patient.

TABLE V. PROTHROMBIN TIME RESPONSE TO VARIOUS CONCENTRATIONS OF PURIFIED PROTHROMBIN*

CONCENTRATION OF ADDED PURIFIED PROTHROMBIN (MG. %)	OLD PLASMA (SEC.)	DICUMAROLIZED PLASMA	J. L.'S PLASMA
	(Control 15.5)	(Control 16)	(Control 16)
0	49	30	53
1	46.5	30	42
2.5	39	29	34.5
5	33.5	28	27.5
10	28.5	25.5	21.5
15	24.5	20.5	19.5
20	22.5	23.5	16.5
100	15	15.5	13.5

*Supplied through the kindness of Dr. Walter H. Seegers, Wayne University Medical School, Detroit, Mich.

TABLE VI. CLOTTING TIMES OF DECALCIFIED PLASMA TREATED WITH DECREASING DILUTIONS OF THROMBIN

THROMBIN DILUTIONS	CLOTTING TIME (SEC.)	
	NORMAL	J. L.
Full strength*	3.5	3
1:2	4	3.5
1:5	4.5	5
1:10	13.5	10
1:20	20	17
1:40	47.5	29.5
Prothrombin time	15	53.5

*Diluted solution of thrombin, topical, Parke, Davis & Company, Detroit, Mich.

Purified prothrombin was added to portions of the respective plasmas to make 100 mg. per cent concentrations. Then a series of dilutions with the proper untreated plasmas produced several concentrations of the added prothrombin. Quick prothrombin estimations were made on the resulting prothrombin solutions. The results are summarized in Table V. Comparison shows that our patient's plasma responded to added prothrombin as well as and in a manner similar to plasma made hypoprothrombinemic by oxidation or dicumarolization. The defect, therefore, did not seem to be an interference with prothrombin conversion.

3. *Thombin Clotting Study:* Defects in the second phase of coagulation can cause a prolongation of the prothrombin time. These may be anticoagulants

*Supplied by Dr. Walter H. Seegers.

which inhibit the effect of thrombin; or the fibrinogen may be qualitatively defective or markedly diminished as in afibrinogenemia.[15] As noted, the quantity of fibrinogen in our patient was normal. Anticoagulant effect and/or fibrinogen defect may be detected by clotting decalcified plasma with increasingly dilute solutions of thrombin.[15] Table VI, summarizing the results of such a study, reveals no evidence of an anticoagulant, or a defect in fibrinogen. Such abnormalities were thus not the cause of the delayed prothrombin time in our patient.

4. *Another Test for Anticoagulant:* If plasma, presumed to be hypoprothrombinemic, contains an anticoagulant it should prolong the prothrombin time of a control beyond that expected by dilution. However, several tests showed that J. L.'s plasma did not prolong the time of the control as much as did the addition of saline. A typical experiment resulted as follows: control plasma was 12 sec.; control plus an equal amount of saline gave 16.5 sec.; control plus J. L.'s plasma in equal amounts was only 13.5 sec. Again an anticoagulant could not be demonstrated in the blood.

5. *Two-Stage Prothrombin Determination:* To help determine whether a retarded conversion rate caused the delayed prothrombin time, the procedure was done in two stages as advocated by Warner, Brinkhous, and Smith.[19] Prothrombin times (Quick) on control and J. L.'s plasmas were 18 and 60, respectively. The plasmas were defibrinated by the addition of thrombin solution. Part of the defibrinated control plasma was diluted 1:3. One-tenth cubic centimeter of each of the three plasma samples was incubated for seventy seconds with $CaCl_2$ (0.1 c.c. of 0.25M) and thromboplastin (0.1 c.c.) solutions. Fibrinogen solution (0.1 c.c.) (Cohn's Human Fraction I used as source) was added to each and the clotting times were noted. These were 16.5, 41.5, and 58 seconds for control, diluted control, and J. L.'s plasmas, respectively. These results would indicate that our patient's deficiency was lack of prothrombin and not a delayed rate of convertibility.

Special Examinations of Plasma Proteins.—(1) Electrophoretic studies of J. L.'s plasma* in 1945 revealed no significant abnormalities. (2) A cryoglobulin was found in abnormal amount. The characteristics of this protein are reported elsewhere.[20] It may be mentioned here, however, that this cryoglobulin was of unusual interest in that, unlike others which have been studied, it contained a carbohydrate fraction.[20] (3) The quality of the fibrinogen was found to be normal by thrombin clotting as detailed previously. In addition, however, the effect of addition of fibrinogen was tested. Bovine fibrinogen,† found to be normally active by thrombin clotting, was dissolved in control and J. L.'s plasmas to make a concentration of 0.5 per cent. The prothrombin times were as follows: control, 15 sec.; control plus fibrinogen, 15 sec.; J. L., 53.5 sec.; J. L. plus fibrinogen, 48 sec. This apparent 5.5 sec. decrease in the prothrombin time was not considered significant.

Component Assay.—Quick[4] has postulated that prothrombin is a complex consisting of calcium, component A, which is an oxidation labile factor, and component B, which is removed by dicumarolization in vivo and by aluminum

*By Dr. H. L. Taylor, Laboratory of Physiological Hygiene, University of Minnesota.
†Supplied by Dr. E. C. Loomis of Parke, Davis & Company, Detroit, Mich.

hydroxide in vitro. More recently Quick[6] speaks of component B as the "conventional prothrombin" and describes a new component A, using the term "labile factor" for the old component A. Seegers and co-workers,[18] however, affirm that prothrombin is composed of but a single component. Deficiency of component B was found in the cases of Lewis and Bennett[11] and of Quick.[4, 5] Owren[13] has described a case which may represent a deficiency in the labile factor.[6] Although at present the exact status of the components is not clear-cut, we attempted to determine the type of component deficiency in our patient. Oxalated plasmas stored approximately one month were used as a source of plasma deficient in the labile factor. Plasma from dicumarolized patients served as a source for component B deficient plasma. The results of the experiments are recorded in Table VII. Since old plasma was more effective than dicumarolized plasma in reducing J. L.'s prothrombin time, it appears that the more important deficiency was of component B. However, the results are difficult to interpret. They may indicate that there was also some deficiency in the labile factor since, in four out of five experiments, stored plasma was not so effective as control plasma in restoring the prothrombin time of J. L.'s plasma. In three of these four experiments, however, dicumarolized plasma plus stored plasma did not result in a normal prothrombin time. Therefore, the possibility exists that there was a deficiency of some other unknown factor.

TABLE VII. PROTHROMBIN COMPONENT ASSAY

	PROTHROMBIN CLOTTING TIME (SEC.)				
	3/25/47	6/12/47	6/19/47	7/2/47	7/3/47
Control	14.5	14.5	15	14	12
Stored plasma	34	67	61	54	67.5
Dicumarolized plasma	31.5	40	43	54.5	38.5
J. L.'s plasma	37	39.5	53.5	53.5	52.5
J. L. + control	17	15.5	16.5	16.5	13.5
J. L. + stored plasma	21.3	17.5	16.5	21.5	15.5
J. L. + dicumarolized plasma	24	26.5	20	36	29
Control + saline				18	16.5
Control + stored plasma		15.5	15	17	16
Control + dicumarolized plasma		18	16	17	14
Dicumarolized plasma + stored plasma		18	14.5	21	16

Response to Vitamin K.—Throughout the patient's course vitamin K and various synthetic vitamin K products in usual doses were tried without effect. The first massive dosage of synthetic vitamin K was given in 1944. Over a three-day period the patient received intravenously a total of 800 mg. of K_1 oxide.* This was associated with a rise in prothrombin time from 70.5 to 102 seconds; the controls had a time of 17 seconds. Recently, six days after a plasma infusion, 72 mg. of menadione bisulfite were administered intravenously. The prothrombin time rose from 34 to 51.5 seconds (controls, 12 and 13 seconds) over a twenty-four hour period. This demonstrates that the patient continued to be vitamin K resistant.

*This study was made by Dr. Rudolf Marshall, at that time a Fellow in Medicine, University of Minnesota Hospital.

Response to Plasma.—Evidence of consistent therapeutic response to blood or plasma transfusions was recorded throughout the entire course of observation. The prothrombin times regularly decreased following such infusions. A few of the more recent examples are summarized in Table VIII. It will be noted that plasmas ten, fourteen, and thirty days old were about as effective as those only three days old. This is confirmatory evidence that the patient's main deficiency was not of the labile factor. Also this observation is of considerable practical importance since stored plasma is readily available in blood banks, whereas there may be some difficulty in obtaining immediately fresh plasma or blood. Pooled dried plasma, because it has been a source of the hepatitis virus, was not given a trial in our patient.

TABLE VIII. PROTHROMBIN TIME RESPONSE TO PLASMA OF VARIOUS AGES AND QUANTITIES

DATE	NORMAL	J. L.'S PROTHROMBIN TIME BEFORE PLASMA	PLASMA (C.C.)	AGE OF PLASMA (DAYS)	J. L.'S PROTHROMBIN TIME AFTER PLASMA
12/30/46	13	35	250	30	21.5
4/ 4/47	13.5	55	180	14	31.5
4/ 7/47	14.5	45	250	10	21.5
5/26/47	12	56.5	250	3	26
6/25/47	12.5	47.5	230	3	24
7/ 3/47	12	52.5	100	3	34
"	50% solution* 16.5	34	100	3	28.5
		28.5	50	3	29
7/14/47	14.5	59	100	3	45.5
	50% solution* 22	45.5	100	3	36
	30% solution* 35	36	100	3	33.5
	20% solution* 50	33.5	150	3	30
	10% solution* 145				

*Dilution of plasma with physiological saline.

The bleeding and clotting times seem to respond favorably with the plasma injections. For instance, on June 19, 1947, the venous clotting time fell from 39 minutes to 15 minutes after 250 c.c. of plasma. The bleeding time (Duke) decreased from 9 minutes 45 seconds to 6 minutes 30 seconds. The prothrombin time diminished from 53.5 to 24 seconds (control, 15 seconds). On June 3, 1947, the ear lobe puncture continued to bleed until the plasma infusion was completed.

Capillary Examination.—Microscopic observation of the capillaries of the nailbed was made.* Many so-called abnormal formations were observed but not in larger proportion than often seen in normal persons. Blood flow was normal in rate and appearance. Traumatization as suggested by Macfarlane[21] showed the injured capillary loop to disappear in normal fashion. Our patient's capillaries, therefore, were normal insofar as this type of examination can indicate.

Studies on Available Family Members.—These studies are summarized in Table IX. Only the mother and the patient noted easy bruising. However, all members except the father showed prolongation of the prothrombin time. Although detailed assay studies were not done, addition of control plasma to plasmas from three of the family resulted in the same type of response as shown with the patient's plasma. Presumably they all had the same type of defect but in different degrees. The sister had a positive cuff test but no history of

*By Dr. R. C. Cullen, Fellow in Medicine, University of Minnesota Hospital.

TABLE IX. PROTHROMBIN AND OTHER DATA IN THE IMMEDIATE MEMBERS OF THE FAMILY OF J. L.

	AGE (YR.)	DATE	CONTROL (SEC.)	50% SALINE SOLUTION OF CONTROL (SEC.)	PROTHROMBIN TIME (SEC.)	50% SOLUTION WITH CONTROL (SEC.)	50% SALINE SOLUTION (SEC.)	BLEEDING TIME	CLOTTING TIME	CLOT RETRACTION	CUFF TEST	EASY BRUISING
Mother	45	7/ 8/47	12	17.5	16	14	22	Normal	Normal	Normal	Positive	Yes
Father	55	7/ 8/47	12	17.5	12		19	Normal	Normal	Normal	Negative	No
Sister	26	7/14/47	14.5	21.5	16	15.5	26	Normal	Normal	Normal	Positive	No
Brother T.	22			*Not available for study*								No
Patient	20	7/ 3/47	12	16.5	52.5	13.5		6/12/47 Normal	Prolonged	Normal	Negative	Yes
Brother R.	17	7/ 2/47	13	18	17			6/19/47 Normal	Normal	Normal	Negative	No
Brother D.	13	7/14/47	14.5	21.5	18	15.5	32	6/11/47 Normal	Normal	Normal	Negative	No

bruising. However, it may be of significance that her 2-year-old daughter was said to bruise easily; so far she had had no episodes of bleeding. These examinations certainly establish the familial nature of the disease in our patient.

DISCUSSION

The history and findings of a patient with idiopathic, familial hypoprothrombinemia have been presented. The patient was followed in detail over a ten-year period. There was a variety of studies and therapeutic attempts in order to ascertain the exact pathologic mechanism. All of the known factors other than hypoprothrombinemia which might give rise to a delay in prothrombin time were presumably excluded by the experiments detailed. There was an evident lack of the prothrombin factor which is decreased by Dicumarol (component B). There was no response to vitamin K, while plasma of various ages was consistently effective. The regular occurrence of a positive cephalin flocculation test, albeit in varying intensity, together with the presence of a cryoglobulin in the patient's serum tends to support the concept of a primary disturbance of protein synthesis in the liver.

The most important result of our studies was the evidence of familial incidence of the disorder. Those afflicted seemed to have the same type of deficiency. This is the fifth reported instance of familial hypoprothrombinemia. Actually, the incidence is probably higher than this would indicate. Reference to Table I shows that in most cases there was a familial hemorrhagic tendency, but prothrombin estimations were not always made. Moreover, just a statement of a negative family history is not sufficient, since several persons with no hemorrhagic manifestations have been found to be mildly hypoprothrombinemic. This was true in the family of J. L. Whenever the diagnosis of idiopathic hypoprothrombinemia is entertained, prothrombin determinations should be made on all available family members to help clarify the diagnosis.

SUMMARY AND CONCLUSIONS

A case of idiopathic hypoprothrombinemia followed in detail for a decade has been presented. The hemorrhagic disease in this case was characterized by epistaxis, subcutaneous hematomas, hemorrhages in proximity to various joints, menorrhagia and metrorrhagia, the latter being so severe as to require hysterectomy.

The familial character of the disease has been established in this instance.

The major deficiency was of the Quick B component, that affected by Dicumarol, yet vitamin K in large amounts was ineffective in shortening the prothrombin time. Purified prothrombin and human plasma, either old or fresh, were the only materials effective in this regard in vitro; the former was not used in vivo, while the latter was consistently effective in controlling the hemorrhagic tendency.

Positive cephalin flocculation and the presence of a cryoglobulin suggest that there may have been a primary disturbance of protein synthesis in the liver.

It is a pleasure to acknowledge the helpful advice and criticisms of Dr. Armand Quick during the course of this study.

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OBSERVATIONS ON ALCOHOLIC FATTY LIVER: THE USE OF INTERVAL NEEDLE BIOPSY AND LIVER FUNCTION TESTS

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INTRODUCTION

DURING the past eight years, needle (trocar) biopsy of the liver has become a recognized procedure in many clinics. Its value in clinical investigation and in the diagnosis of obscure cases of liver disease is well established.

This is a report of observations on seven cases of so-called alcoholic liver. Biopsies give evidence of rapid disappearance of fat following therapy in five of these cases. An attempt will be made to correlate the more important liver function tests with these changes, along with the clinical features of the cases and the response to therapy.

Hoffbauer[1] recently gave a summary of the different techniques and of the indications for and dangers and advantages of needle biopsy. In a series of 1,200 cases, Sherlock[2] reported a mortality of 0.67 per cent. Hemorrhage is by far the most common cause of death. Other disadvantages include the failure always to secure an adequate specimen, and the possibility that one shred of tissue may not represent accurately the picture of the rest of the liver. However, the needle biopsy usually does offer a more representative picture than the ordinary surgical biopsy because the latter penetrates only a small distance through the capsule.

The pathogenesis of fat infiltration in the liver has been studied extensively in animals in recent years. By the use of serial needle biopsies the pathogenesis of the so-called fatty liver in man should now be amenable to investigation.

Experimentally, fatty livers may be divided into two types[4]: those produced by an increase in the rate at which fat is supplied to the liver, and those produced by a decrease in the rate at which the liver is able to dispose of fat. In the first category belongs the fatty liver produced by starvation, high fat diet, and stimulation with the ketogenic fraction of the anterior lobe of the pituitary.[5] The second group includes fatty livers resulting from poisoning with carbon tetrachloride or phosphorus, depancreatization plus insulin (dogs), and choline deficiency combined with a low protein diet. Some investigators[6] believe that cirrhosis in man, as in certain experimental animals,[7] may be the end result of extensive fat infiltration.

Incipient cirrhosis in alcoholics often is characterized by fatty infiltration and enlargement of the liver.[8] It is in such cases (as shown below) that spontaneous remissions occur when alcohol is withdrawn and a satisfactory dietary regime is established. Treatment does not cause disappearance of the fibrosis, but presumably does aid in liver cell recovery and regeneration.

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Received for publication, Oct. 17, 1947.

In clinical therapeutic studies with fortified diets of various types, it has been noted that the prognosis in cirrhosis seems related to liver size.[9, 10] Few subjects with small livers did well with any therapy, whereas those with large, easily palpable organs improved, presumably because of resorption of liver fat. Evidence is presented here that this is indeed the case.

MATERIAL AND METHODS

During the year ending in June, 1947, a total of fifty-six needle liver biopsies on twenty-eight patients has been attempted at the Salt Lake Veterans' Hospital and the Salt Lake County General Hospital. There were twenty-two failures (due to insufficient tissue for making a microscopic diagnosis). The Roth-Turkel needle was used in all instances and the technique as described by Davis and co-workers[3] was followed. When the liver was easily palpable, it was usually approached from below the right costal margin anteriorly. An intercostal approach in the anterior or posterior axillary line was used when the liver was not palpable.

Only the liver function tests used routinely in these selected cases are recorded in Tables I and II. These were done by generally recognized methods.[11-14]

Emphasis will be placed here on a selected group of seven white, male alcoholics with fatty livers. In Tables I and II a summary of the important clinical features, liver function tests, histologic observations, and treatment of these subjects is given.

Two needle biopsies were performed on each of the seven patients, the first soon after admission and the second after an interval of from three weeks to three and one-half months. The first five cases summarized in the tables had a favorable outcome. Patient B. N. (Case 6) was committed to the state mental hospital where he expired within a month. Autopsy was not performed. Case 7 is included to contrast the course with that of the first five cases and also because the immediate cause of death was hemorrhage following a needle biopsy. One other complication occurred in the total series—a right pneumothorax. Recovery was uneventful.

CLINICAL OBSERVATIONS

All of these patients gave evidence of prolonged and severe alcoholism on admission to the hospital. The salient symptoms and signs for each are listed in Table I. They were the usual findings in varying degrees of decompensated cirrhosis and nutritional deficiency.

LABORATORY FINDINGS

The laboratory data before treatment indicated mild to moderate hepatic insufficiency. The cephalin-cholesterol flocculation test was initially positive in three of the seven cases. Of the five cases in which the thymol turbidity was measured on admission, the findings were abnormal in only two. Moderate to marked hypoalbuminemia was present in five of the seven subjects. There was bromsulfalein retention in all the patients and four had an initial mild anemia of the macrocytic type.

HISTOLOGIC OBSERVATIONS

The needle liver biopsy, done on all patients at the time of admission, showed moderate to marked diffuse fatty infiltration and from normal to moderate increase in fibrous tissue in the periportal areas. Inflammatory cells, mostly lymphocytes, were present in varying numbers at the triads. Scattered areas of degeneration of liver cord cells were observed in three patients on admission.

TABLE I. CLINICAL DATA BEFORE AND AFTER TREATMENT

CASE	PATIENT	TIME INTERVAL (WK.)	JAUNDICE	LIVER SIZE*	ASCITES	ANKLE EDEMA	OTHER MANIFESTATIONS	THERAPY
1	H. K.	4	++	7	+	++	Peripheral neuropathy; delirium tremens	Diet† and B complex vitamins, after 1,000 c.c. Amigen and 1,000 c.c. 10 per cent glucose daily for 10 days
			0	1	0	0	Asymptomatic; gained 30 pounds	
2	W. S.	6	+++	10	++	0	Acutely ill; mentally obtunded; spider naevi	300 c.c. Parenamine in 1 liter 5 per cent glucose daily for 1 week plus multivitamins; then diet plus 300 c.c. Parenamine orally daily
			+−	6	0	0	Much improved; gained 18 pounds	
3	A. G.	4	0	4	+	+	Tremor of hands; not acutely ill	House diet; no other vitamins or other supplemental therapy
			0	1	0	0	Asymptomatic; lost 10 pounds	
4	W. O.	4	+	4	0	0	Acutely ill	Diet† only; no supplementary vitamins
			0	1	0	0	Asymptomatic; no change in weight	
5	H. P.	14	0	3	0	0	Delirium tremens; peripheral neuropathy	In hospital (8 weeks); diet,† B complex vitamins, plus 90 c.c. Pro-colysate per day
			0	Not palpable	0	0	Asymptomatic, except neuropathy unchanged	Out patient (6 weeks); no special diet; no alcohol
6	B. N.	3	0	8	++	0	Korsakow's psychosis	Diet†; did not eat well; choline 4 Gm. per day
			0	8	+	0	Unimproved; died in another hospital	
7	F. W.	13	0	4	0	0	None	Diet† and B complex vitamins. Improved; in hospital 2 weeks; out of hospital 10 weeks; did not abstain from alcohol; died following liver puncture
			++++	9	+++	+	(Third admission.) Stuporous, critically ill	

*Liver size is in centimeters below the right costal margin in the midclavicular line.
†Diet was composed of protein, 150 Gm.; carbohydrate, 300 Gm.; fat, 70 Gm.

Treatment consisted essentially of rest, withdrawal of alcohol, a high protein, high carbohydrate, and low fat diet, and B complex vitamins.

OBSERVATIONS AFTER TREATMENT

Results of treatment can be summarized as follows.

1. Rapid clinical improvement (first five cases) such as gain in appetite, strength, and weight, diminution in liver size, edema and ascites, and clearing of icterus in the jaundiced patients.

2. Consistent and significant increase in serum albumin.

3. Return of hemoglobin to normal.

TABLE II

CASE	PATIENT	TIME INTERVAL (WK.)	LABORATORY DATA: THYMOL TURBIDITY (UNIT)	CEPHALIN FLOCCULATION	SERUM ALBUMIN (GM./100 C.C.)	SERUM GLOBULIN (GM./100 C.C.)	BSP RETENTION (%)	VAN DEN BERGH (1 SEC.)	TOTAL VAN DEN BERGH	HEMOGLOBIN (GM./100 ML.)	HISTOLOGIC DATA: LYMPHOCYTIC INFILTRATION	FAT	PERIPORTAL FIBROSIS
1	H. K.	4	—	3+	3.6	2.6	29	1.6	12.0	12.0	+	3+	2+
			6.0	3+	4.2	3.5	6	.6	1.6	13.5	+	1+	2+
2	W. S.	6	7.0	4+	3.0	3.5	—	2.5	27.0	12.5	+	4+	2+
			6.5	Neg.	4.1	4.2	15	.3	4.1	16.0	4+	1+	3+
3	A. G.	4	3.0	Neg.	5.3	2.6	21	.3	1.2	15.0	+	4+	1+
			5.0	Neg.	—	—	15	.1	1.0	15.0	+	1+	0
4	W. O.	4	16.5	4+	3.7	1.9	18	.8	8.0	12.3	+	2+	1+
			9.0	Neg.	4.8	2.0	8	.3	1.2	15.0	2+	0	1+
5	H. P.	14	2.5	Neg.	4.0	3.3	23	.5	1.4	15.0	2+	3+	2+
			2.5	Neg.	4.1	3.3	17	.3	.9	17.0	3+	0	2+
6	B. N.	3	—	Neg.	4.1	2.8	—	.4	.9	11.0	2+	3+	2+
			—	—	—	—	—	—	—	—	+	2+	2+
7	F. W.	13	4.0	—	5.4	2.2	40	.8	2.4	18	+	1+	2+
			8.0	3+	2.5	3.5	—	8.4	35.0	14.5	Polys* 4+	1+ Widespread necrosis	1+

—, Tests were not done.
1+, Approximately amount seen in Fig. 4; 4+, illustrated by Fig. 3.
*Polymorphonuclears.

4. Reversion of the cephalin-cholesterol flocculation to negative in those cases in which it was positive.

5. In contrast, only in one case out of the five in which two tests were performed was significant improvement in the thymol turbidity observed.

6. Decrease in the quantity of bilirubin in the serum in the jaundiced patients with corresponding change in the bromsulfalein test. The latter returned to normal in two instances.

7. Histologically there was a consistent and marked diminution in fat in the first five subjects without marked change in periportal fibrosis and signs of inflammation. Many cells showed double nuclei. This was interpreted as evidence of regeneration.

COMMENT

It was pointed out by Hoffbauer, Evans, and Watson[15] that it is not always possible to correlate closely liver function tests and anatomic findings. The same impression is gained here in cases of fatty liver. The bromsulfalein retention test and the serum albumin level seemed to be the most consistent in reflecting the clinical and histologic status before and after treatment.

It may be noted (see Table II) that the Hanger test may be negative when the liver is fatty. Thymol turbidity was variable and not markedly elevated. Similar results for these tests in cirrhosis have been noted before.[16, 17]

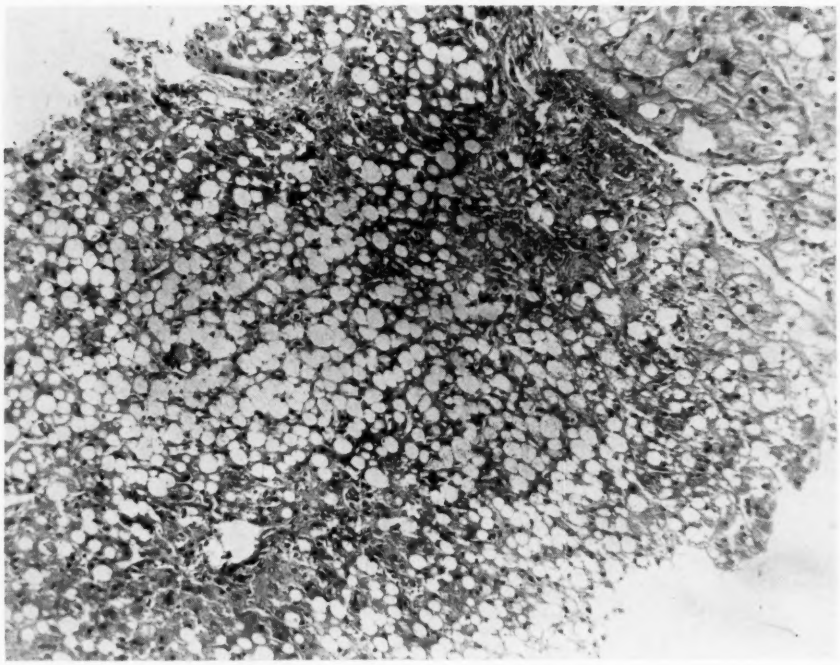

Fig. 1.—Case 2. Specimen taken on admission showing marked fatty infiltration and slight periportal fibrosis and lymphocytic infiltration (low power; hematoxylin and eosin).

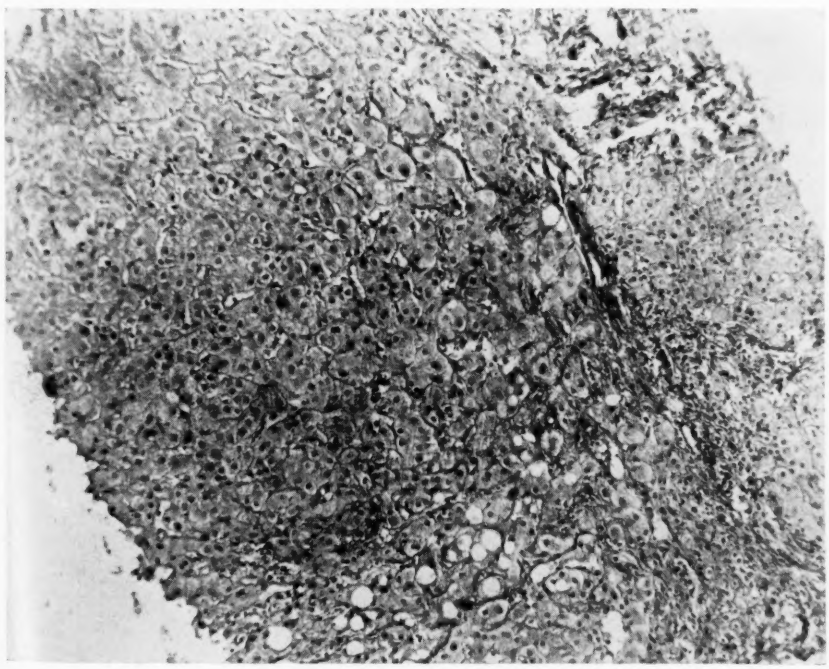

Fig. 2.—Case 2. Specimen taken six weeks later showing marked diminution of fat with apparent increase in fibrosis and lymphocytes (van Gieson).

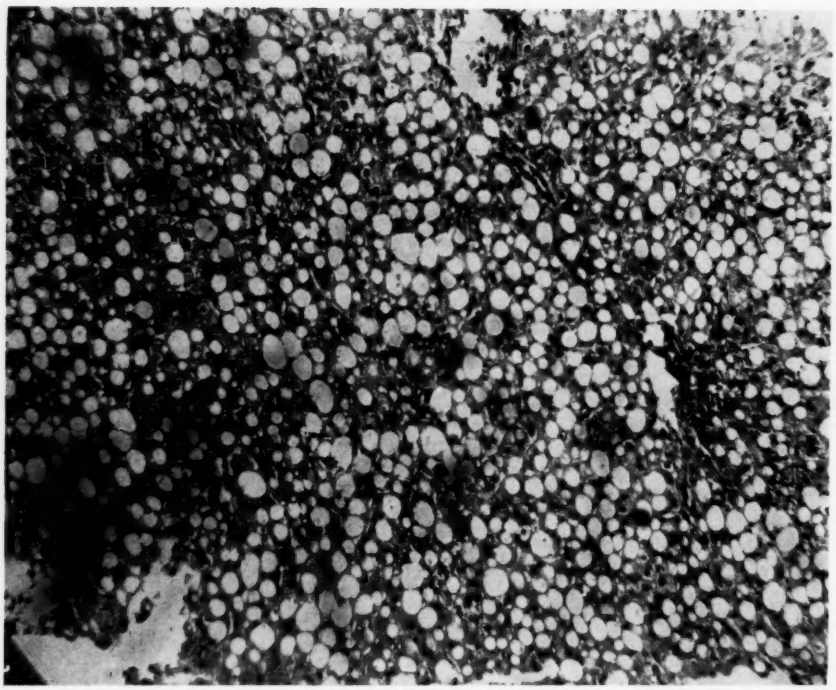

Fig. 3.—Case 3. Specimen taken on admission showing marked fatty infiltration (hematoxylin and eosin).

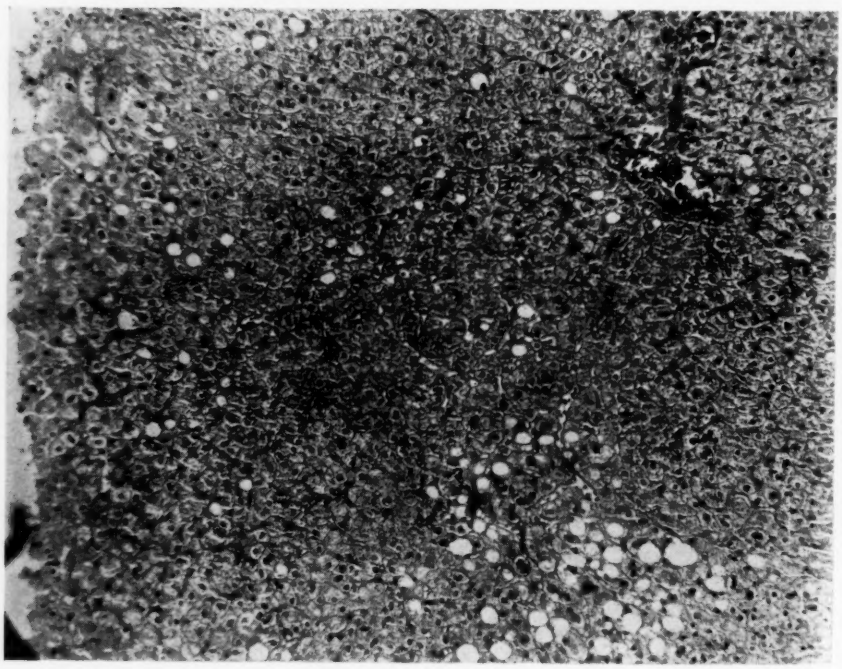

Fig. 4.—Case 3. Specimen taken four months later demonstrating marked decrease in fat (hemotoxylin and eosin).

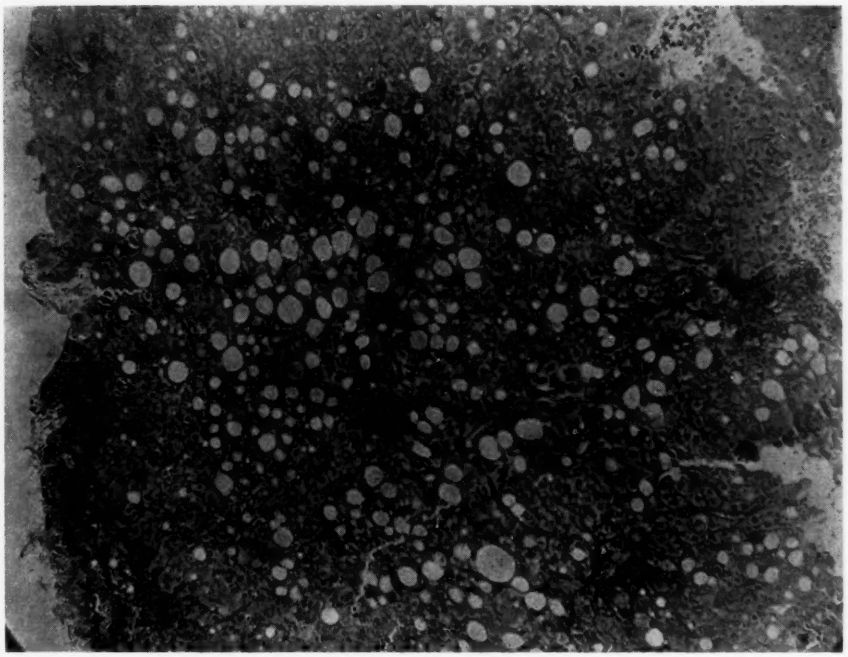

Fig. 5.—Case 7. Specimen taken thirteen weeks before death showing moderate increase in fat and early cirrhosis (van Gieson).

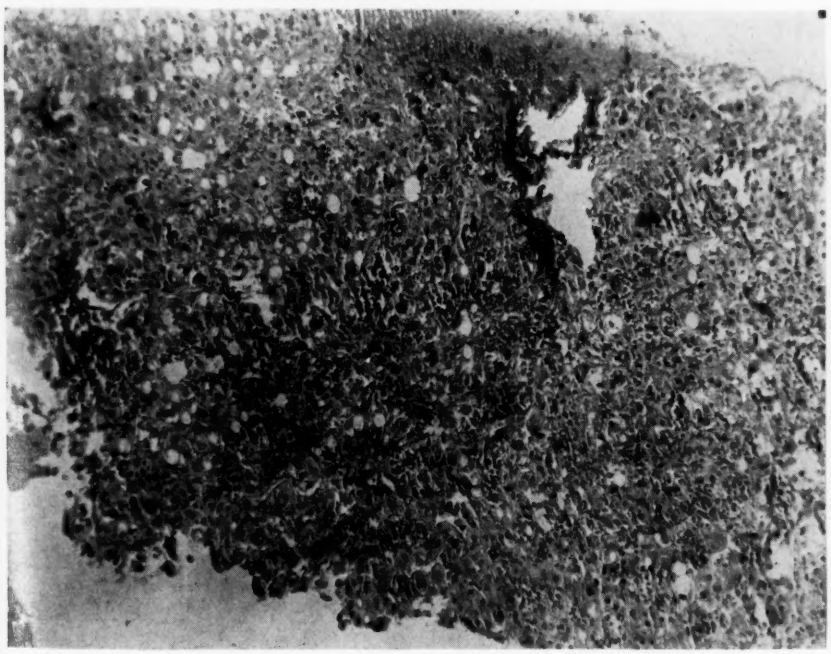

Fig. 6.—Specimen taken on day of death (Case 7) showing cellular necrosis and dense polymorphonuclear infiltration (van Gieson).

The finding of principal interest was the anatomic evidence of rapid diminution of fat in the first five cases. It seemed remarkable that the liver could dispose of what appeared to be an enormous amount of fat in the short time interval of four weeks (in three cases). (See Figs. 1 to 4.) Presumably the processes concerned in lipid transport from the liver become effective soon after the initiation of therapy. The type of treatment seemed to make no particular difference as judged by the clinical result, function tests, and biopsy changes.

Two patients received amino acids parenterally as well as B complex vitamins and glucose intravenously until their appetites returned. Then they were given a diet of protein, 150 Gm., carbohydrate, 300 Gm., and fat, 70 grams. Patient A. G. (Case 3) received the house diet only (no vitamins or supplementary nourishment) and yet made a rapid recovery as judged clinically and by laboratory tests. In contrast to this, Patient B. N., who was psychotic and ate poorly but received 4 Gm. choline per day, showed no appreciable change in liver fat over a three-week period.

In the course of this study, one fatality occurred as a consequence of liver biopsy. Patient F. W. (Case 7, Tables I and II) expired three hours following the second (interval) liver puncture. This admission was his third within a year for liver disease. The patient was critically ill, with the signs and symptoms of acute liver failure. At autopsy death was shown to be due to hemorrhage from the liver biopsy wound and there was widespread necrosis and hepatitis superimposed on a fatty cirrhosis (Figs. 5 and 6).

Liver puncture in this case was performed in the face of two contraindications: a prothrombin time of 50 per cent, not affected by vitamin K, and a patient too ill to cooperate. Death occurred without warning by any signs or symptoms of hemorrhage.

DISCUSSION

It appears that a good clinical result is obtainable in cases of fatty liver as long as the patient can eat, rest, and abstain from alcohol. It seems doubtful that the addition of lipotropic agents would have altered the course in the cases discussed since the rate of disappearance of abnormal amounts of lipid, in at least three of the cases, would seem to be difficult to improve upon.

The clinical syndrome of an alcoholic who enters the hospital acutely ill with jaundice, edema, ascites, and hepatomegaly is usually classified as cirrhosis of the liver. Actually all these symptoms or signs of hepatic decompensation can be produced by marked fatty infiltration and cellular damage with very little actual cirrhosis. Death may occur suddenly and at autopsy little or no trace of cirrhosis may be found.[21, 22] Or, as reported here in the first five cases, rapid, almost complete recovery may take place. Fatty changes and the accompanying signs of hepatic insufficiency may closely simulate those of cirrhosis, yet the process is usually rapidly reversible with appropriate dietary therapy plus abstinence from alcohol.

It may be pointed out that treatment is the same regardless of the degree of cirrhosis present. The need for obtaining a picture of the histologic appearance of the liver may therefore be questioned. However, the initial pathologic

finding of only fat infiltration and the later demonstration of a relatively normal liver can be a powerful stimulus in indicating the value of an attempt at rehabilitation of the patient. The usual alcoholic relapse on discharge from the hospital might be averted if the patient and his physician had a clear picture of the changes in the liver associated with therapy and abstinence from alcohol and poor dietary habits. Resumption of former habits almost certainly would result in another episode of liver disease, whereas with abstinence, a lifetime free from liver disease can at least be hoped for.

SUMMARY AND CONCLUSIONS

Experience with fifty-six attempts at needle puncture biopsy of the liver (including one pneumothorax and a death from hemorrhage) is reported.

Follow-up biopsies performed in seven patients with alcoholic liver at intervals of from three weeks to three and one-half months are presented, as well as simultaneous liver function studies and clinical findings.

Clinical improvement (coinciding with rapid diminution in the quantity of liver fat) was noted in five patients, and similar improvement was observed in liver function tests. In patients who could eat an adequate diet, the use of supplementary lipotropic agents seemed unnecessary.

Fatty liver with a mild underlying cirrhosis may exhibit all the clinical signs of an advanced portal cirrhosis and is usually diagnosed as such. Differentiation is important in prognosis.

It is believed that if the procedure is carried out by the fewest possible operators and if due attention is given to the contraindications for the procedure, liver puncture is justified in selected cases for diagnosis and prognosis. This does not imply that it should be a routine method in the clinical diagnosis of liver disease.

Serial biopsies in early cirrhosis over a period of years would add much in elucidating the natural history of the disease.

The author acknowledges the aid and advice of Dr. B. V. Jager and Dr. F. D. Gunn.

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THE THYMOL TURBIDITY TEST IN VARIOUS DISEASES

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THE thymol turbidity test was first described by Maclagan[1, 2] in 1944 as an index of liver function. Since then many papers have appeared in the literature concerning its value. The exact mechanism of the test has not as yet been completely determined. The turbidity is due to the formation of a complex consisting of a globulin, phospholipid, cholesterol, and thymol. Maclagan[2] believed that the globulin was gamma globulin and that the thymol turbidity and cephalin flocculation tests had a similar mechanism. Recant and co-workers,[3] using electrophoretic methods, were able to show that gamma globulin was not involved in the mechanism of the thymol turbidity test, while the cephalin flocculation test depended on the presence of gamma globulin. Recently Cohen and Thompson[4] presented evidence that the protein in the complex of the positive thymol turbidity test was beta globulin. Clinically, investigators[5, 6] have felt that the basic mechanisms of the thymol turbidity test and the cephalin flocculation test were different. Kunkel and Hoagland[7] have offered experimental evidence to show that the development of the turbidity depends on both lipids and gamma globulins in the serum. However, the lipid protein complex migrates in the beta globulin fraction of the serum.

The thymol turbidity test has many advantages over other tests of liver function in current use, that is stability of reagents, quantitative method of determination, short interval of time needed for performance, and apparent sensitivity. It was therefore decided to evaluate this procedure by testing sera from patients with liver disease as well as various other diseases.

MATERIALS AND METHODS

The technique used for the performance of the thymol turbidity test was the modification suggested by Shank and Hoagland.[8] Sera showing 5 or more units were classed as abnormal. This was based on our own observations of the test and on the investigations of Hoagland and Shank. These workers used 4.7 units as the upper limit of normal. A cephalin flocculation test was performed on each serum at the same time that the thymol turbidity determination was done. The cephalin flocculation test was read at the end of forty-eight hours and was called positive if it read 3 plus or 4 plus. In most instances total protein and formol gel determinations were also carried out. Bromsulfalein tests were done when indicated.

Although many of the patients tested were thought to have liver dysfunction, a large number of tests were made on patients in whom liver disease was not thought to be present. Many patients were tested repeatedly.

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Received for publication, Feb. 4, 1948.

RESULTS

Tests were carried out one or more times on 567 persons. Seventy-four of these were regarded as normal controls. The results are presented in Table I, in which an attempt has been made to classify the cases. It will be noted that a positive result was obtained in 295, or 52 per cent of all persons tested.

Certain comments may be made regarding the results in the different groups.

TABLE I

GROUP	NUMBER OF CASES	NUMBER T.T.+	PER CENT T.T.+	POSITIVE THYMOL TURBIDITY TESTS	
				AVERAGE NUMBER OF UNITS	RANGE
Infectious hepatitis	36	34	94.5	23	5 - 50
Cirrhosis of the liver	48	46	96	20	5 - 49
Obstructive jaundice	12	5	42.5	17	6½ - 38
Diseases of the gall bladder	11	6	54.5	8	5 - 20
Weil's disease	4	4	100	17.4	8 - 28½
Diseases with widespread liver destruction	11	8	73	14	6 - 33½
Neurosyphilis with fever therapy	9	9	100	19	8 - 28
Neurosyphilis without fever therapy	9	2	22.2	10.3	6½ - 14
Acute lymphogranuloma venereum	21	20	95.3	13.6	5 - 35
Acute and chronic rheumatoid arthritis	17	14	82.4	10	5 - 19
Acute rheumatic fever	11	6	54.5	6.7	5 - 9
Congestive heart failure	56	27	48	8.4	5 - 25
Heart disease without failure	19	9	47	8.4	7 - 15½
Chronic lung disease	28	15	53.5	11	5 - 26
Acute infectious diseases	67	25	37.3	9	5 - 53
Neoplastic disease	15	7	46.5	9.5	5 - 16
Diabetes mellitus	19	6	31.6	8.6	5½ - 10½
Ulcers and gastrointestinal hemorrhage	18	3	16.7	8	6½ - 11½
Thyrotoxicosis	7	4	57	9.8	5½ - 14
Nutritional disease	10	6	60	8	5 - 16½
Chronic ulcerative colitis	3	2	67	9	6 - 12
Amebiasis	4	0	–	–	–
Hemolytic crises	5	4	80	8	6 - 11½
Chronic alcoholism	4	0	–	–	–
Miscellaneous	49	28	57		
Controls	74	6	8	7.5	6½ - 9½
Total	567	295	52		

Infectious Hepatitis.—Sera of thirty-six cases of infectious hepatitis were tested; four of these were probably cases of homologous serum jaundice. All but two of the thirty-six had a positive test, the average number of units being 23. One of the remaining cases had a negative test initially but one week later the result was positive. In the remaining case, which was typical of infectious hepatitis clinically, the thymol turbidity test remained negative throughout the hospital course. These results agree closely with those quoted in the literature.

Cirrhosis of the Liver.—There were forty-eight subjects in this series and forty-six of them gave a positive thymol turbidity test. The average number of units was 20. A study of our cases revealed that the thymol turbidity test showed no essential difference between the cases of cirrhosis which occurred in alcoholics and those which occurred in nonalcoholics. Chronic hepatitis following infectious hepatitis cannot be separated from cirrhosis by the thymol turbidity test.

Of the two patients with cirrhosis with negative thymol turbidity tests on admission, one turned positive a month later while the other remained negative throughout the observation period of two weeks.

Obstructive Jaundice.—Five of the twelve patients in our series had a positive thymol turbidity test. Four of the five patients with positive tests were shown to have cholangitis in the presence of obstruction, and the same condition was suspected in the fifth positive case.

Diseases of the Gall Bladder.—This group of eleven patients included cases of acute and chronic cholecystitis as well as cholelithiasis. In six of them the thymol turbidity test was positive. It seems possible that the positive results were due to an associated cholangitis.

Diseases With Widespread Liver Destruction.—Eleven such cases were tested in the series; these included primary neoplastic disease of the liver, massive metastatic infiltration of the liver, and one case of massive infarction of the liver. Eight of the eleven patients had a positive thymol turbidity test.

Therapeutic Malaria.—Nine patients undergoing malarial fever therapy for neurosyphilis were tested. All of them had strongly positive thymol turbidity tests, with increasing values according to the duration of the fever therapy. This is in agreement with the results of other observers who have noted that other tests of liver function give evidence of liver damage.[9-11]

Acute Lymphogranuloma Venereum.—This group of twenty-one cases was unusual in that the virus of lymphogranuloma venereum had been isolated in every instance.[12] All but one of the patients had a positive thymol turbidity test. Maclagan in his original work included one case of lymphogranuloma venereum, and that patient had a strongly positive thymol turbidity test.

Rheumatoid Arthritis.—Seventeen patients with acute and chronic rheumatoid arthritis were tested. Fourteen of them had a positive thymol turbidity test. The average number of units was 10. Carter and Maclagan[13] obtained positive results in thirteen of thirty-four patients with rheumatoid arthritis.

Acute Rheumatic Fever.—Six of eleven patients with acute rheumatic fever had weakly positive thymol turbidity reactions. The average number of units was 6.7.

Heart Disease With and Without Failure.—Approximately half of the patients with various forms of organic heart disease had positive thymol turbidity tests. The presence or absence of circulatory failure did not appear to influence the result. Carter and Maclagan found that ten of twenty-eight cases, or 36 per cent of their patients with congestive heart failure, had positive thymol turbidity tests.

Chronic Lung Disease.—There were twenty-eight patients in this group which included such entities as bronchiectasis, empyema, pulmonary fibrosis, asthma, chronic emphysema, lung abscess, and chronic pulmonary tuberculosis. Fifteen of them had a positive thymol turbidity reaction. Five of the six patients with bronchiectasis gave positive tests.

Acute Infectious Diseases.—Sera of sixty-seven patients with acute infectious diseases were tested and 37.3 per cent showed a positive reaction. The

incidence seemed notably high in miliary tuberculosis, secondary syphilis, infectious mononucleosis, tularemia, and bacterial endocarditis. The highest value in the entire series, namely 53 thymol turbidity units, was found in the serum of a patient with miliary tuberculosis. Since infectious mononucleosis may be accompanied by a hepatitis it is not surprising to find the thymol turbidity test positive during the acute phase of this disease. Maclagan[2] found that six of seven patients with subacute bacterial endocarditis had a positive thymol turbidity test. Negative results were obtained in such diseases as labor pneumonia, typhus, pharyngitis, tuberculous peritonitis, and so on.

Neoplastic Diseases.—Fifteen patients were studied and seven had a positive thymol turbidity test. This group included three cases of bronchiogenic carcinoma and three of carcinoma of the colon.

Controls.—Seventy-four members of the medical and nursing staffs were included in this group. Six, or 8 per cent, had positive thymol turbidity tests, ranging between 5.0 and 9.5 units. Most other workers[1, 5, 8, 14] have reported that the normal control subjects did not give values above 4.0 to 4.7 thymol turbidity units. Ley and co-workers[15], however, determined their normal values for the thymol turbidity test statistically and concluded that the upper limit of normal was 8.7 units. Using this figure as the maximal normal level, 1.9 per cent of their controls had an elevated thymol turbidity test. It is of interest to note that the thymol turbidity test in 8.5 per cent of their controls exceeded 5 units.

COMPARISON OF THYMOL TURBIDITY TEST WITH CEPHALIN FLOCCULATION TEST

Although there is both experimental and clinical evidence that the thymol turbidity and cephalin flocculation tests depend on different factors, it was of interest to compare the two tests in our series since both are generally regarded as liver function tests. Table II shows the comparative results of the two tests in some of the larger groups of patients in the present series of cases.

TABLE II. COMPARISON OF THE THYMOL TURBIDITY AND CEPHALIN FLOCCULATION TESTS

GROUP	NUMBER OF CASES	T.T. + C.F. +	T.T. + C.F. −	T.T. − C.F. +	T.T. − C.F. −
Infectious hepatitis	36	27	7	1	1
Cirrhosis	48	37	9	1	1
Obstructive jaundice	12	2	3	1	6
Lymphogranuloma venereum	21	19	1	1	–
Neurosyphilis with fever therapy	9	9	–	–	–

Infectious Hepatitis.—Results of twenty-eight of the thirty-six cases agreed in both tests. There were seven tests in which the thymol turbidity was positive and the cephalin flocculation negative and one in which the cephalin flocculation was positive and the thymol turbidity negative. This is in agreement with other investigations, indicating that the thymol turbidity test is more sensitive than the cephalin flocculation test in hepatitis.[2, 16, 17] Repeated examinations of our patients with the two tests also indicated that the thymol turbidity was a better test for following the progress of infectious hepatitis than the cephalin flocculation, since it remained positive as long as there were any symptoms of the disease. An example in a case of infectious hepatitis is illustrated in Fig. 1.

The patient was a 46-year-old white woman who entered the hospital in the acute phase of infectious hepatitis. At the time of entry the thymol turbidity was 43 units and the cephalin flocculation 4 plus. The thymol turbidity fell rapidly. The patient was allowed to be out of bed thirty-five days after admission before the thymol turbidity had reached a "normal" level. Within a few hours she complained of nausea and pain in the right upper quadrant. A thymol turbidity taken at this time showed a rise from 8 to 26 units. Bed rest was resumed and one day later the thymol turbidity fell to 11 units, while the cephalin flocculation was 2 plus. Ninety days after admission the thymol turbidity was normal. This was forty-five days after the cephalin flocculation had become negative.

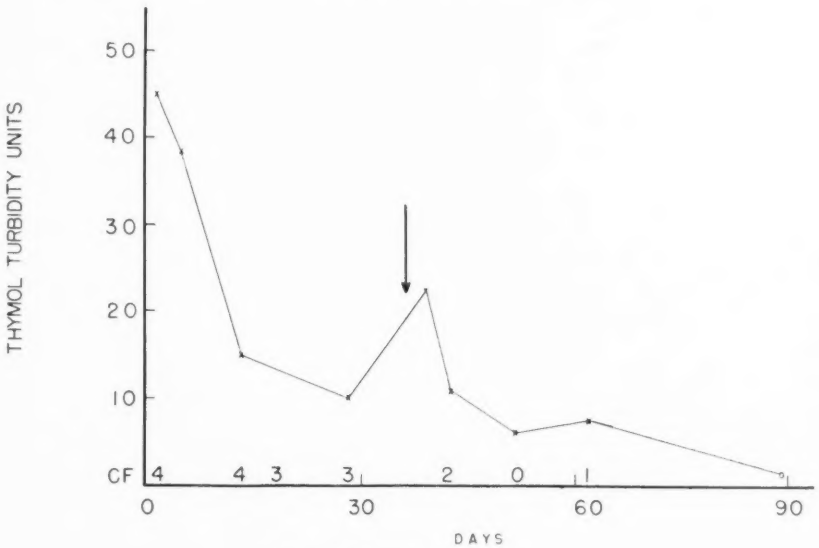

Fig. 1.—Infectious hepatitis.

This case emphasizes the usefulness of the thymol turbidity test in determining the length of convalescence in infectious hepatitis. Correlation with liver biopsy seems to indicate that the thymol turbidity test is the most accurate index of recovery from this disease.

Cirrhosis.—The two tests agree in a majority of the cases. However, here also the thymol turbidity test appeared to be more sensitive than the cephalin flocculation test. There were nine cases in which the thymol turbidity was positive while the cephalin flocculation was negative. In contrast, there was only one case with a positive cephalin flocculation and a negative thymol turbidity test.

Obstructive Jaundice.— In this series of only twelve cases it is not possible to draw conclusions about the relative usefulness of thymol turbidity and cephalin flocculation tests. Neither test can be relied upon to rule out this diagnosis. Their greatest usefulness seems to be in cases of jaundice with a negative thymol turbidity or cephalin flocculation. In such circumstances the chances are against the likelihood of hepatitis. However, a positive thymol turbidity or cephalin flocculation test does not rule out obstructive jaundice. It appeared that the cephalin flocculation was a slightly better diagnostic aid than the thymol turbidity test in this syndrome since the thymol turbidity test was positive in cases with cholangitis in which the cephalin flocculation test was negative.

Lymphogranuloma Venereum.—It should be pointed out that the high incidence of positive results with both tests shown in this report applies to acute cases. It is probable that in chronic lymphogranuloma venereum the incidence of positive tests would not be as great. Nevertheless the possible existence of this disease must be considered when the test is employed in colored patients.

Therapeutic Malaria.—Both tests were positive in all nine cases. The results here are essentially similar to those which have appeared in the literature.

TABLE III. COMPARISON OF THYMOL TURBIDITY AND CEPHALIN FLOCCULATION TESTS

	NUMBER OF CASES	T.T. + C.F. +	T.T. + C.F. −	T.T. − C. F. +	T.T. − C. F. −
Total	567	29%	25%	6%	40%

Table III presents a comparison of the thymol turbidity and cephalin flocculation tests of our entire series. Including the control cases there were 567 cases studied. The thymol turbidity and cephalin flocculation tests agreed in 69 per cent of the cases. The thymol turbidity was positive and the cephalin flocculation negative in 25 per cent of the cases; the thymol turbidity was negative and the cephalin flocculation positive in 6 per cent of the cases. Therefore, it would seem that the thymol turbidity test is considerably more sensitive than the cephalin flocculation test.

TABLE IV. COMPARISON OF THYMOL TURBIDITY AND CEPHALIN FLOCCULATION TESTS IN RARE DISEASES

DIAGNOSIS	T.T.	C.F.
Disseminated lupus erythematosus	23	−
Disseminated lupus erythematosus	16	−
Dermatomyositis	23.5	3+
General myositis	17.5	3+
Scleroderma	21.5	3+
Calcinosis universalis	12.5	−
Pemphigus	10	−
Pemphigus	−	−
Porphyria	−	−
Hypoprothrombinemia	−	−
Elephantiasis	10.5	−

Table IV shows the results of the thymol turbidity and cephalin flocculation tests in some rare diseases. There were two cases of disseminated lupus, both strongly positive with the thymol turbidity and negative with the cephalin flocculation. There was one case each of dermatomyositis, generalized myositis (possibly dermatomyositis, but with a negative skin and muscle biopsy), scleroderma and calcinosis universalis (due to dermatomyositis). Both tests were positive in three of the four cases and in the fourth case (calcinosis universalis) the cephalin flocculation was negative. One case of pemphigus gave a negative result with both tests, whereas in a second case the thymol turbidity was positive and the cephalin flocculation negative. One case of porphyria and one of idiopathic hypoprothrombinemia had negative results with both tests.

COMMENT

It appears from the results just presented that the thymol turbidity test cannot be regarded solely as a test of liver function. Positive results were frequently obtained in diseases in which there was no other evidence of liver dysfunction. Such diseases included rheumatic fever, congestive heart disease, lymphogranuloma venereum, carcinoma without liver involvement, and so on. The thymol turbidity test should be looked upon only as a measure of abnormal serum protein pattern, not necessarily related to liver function. It is conceivable that many conditions other than liver disease might occasion a change in the pattern of the serum proteins. Evidence in favor of this hypothesis is offered by the number of positive thymol turbidity tests in the control series of apparently normal healthy young men and women of the professional staffs.

In cases of liver dysfunction, the thymol turbidity test is a rather sensitive test in such diseases as infectious hepatitis, cirrhosis, Weil's disease, malaria, and so forth. While it is a great aid in diagnosis of these conditions, it can be used to follow the progress only of infectious hepatitis and probably Weil's disease. Labby and co-workers[18] have shown that neither the thymol turbidity nor the cephalin flocculation test is of value in following the progress of cases of cirrhosis. This is unfortunate since the thymol turbidity test is a quantitative test and can be performed easily.

There are certain features of rheumatoid arthritis, that is palmar erythema, the remission of symptoms with the onset of jaundice, and the positive liver function studies,[19] which seem to indicate dysfunction in the liver in rheumatoid arthritis. The high percentage of positive thymol turbidity tests in this disease adds another link in the circumstantial evidence of such a relationship.

It has long been recognized that lymphogranuloma venereum is a generalized disease. The incidence of this disease is rather high among Negroes. Beeson and Miller[20] have shown that many Negroes have abnormal serum protein reactions as evidenced by positive formol gel reactions. They postulated the possibility that these two findings were related. The high incidence of positive thymol turbidity and cephalin flocculation tests in lymphogranuloma venereum tends to confirm this hypothesis.

SUMMARY

The sera of 567 individuals were studied in order to evaluate the thymol turbidity test. The results indicate that the thymol turbidity test should not be regarded as a specific test of liver function. It appears to depend upon abnormal protein patterns which may or may not reflect liver disease. Many conditions in which all other tests of liver function are normal may have a positive thymol turbidity test.

The test is of greatest value in following the progress of cases of infectious hepatitis.

Both thymol turbidity and cephalin flocculation are frequently positive in lymphogranuloma venereum and rheumatoid arthritis, thus necessitating cautious evaluation of the tests in the presence of these diseases.

Negative thymol turbidity and cephalin flocculation tests are helpful in the differential diagnosis between obstructive jaundice and hepatogenous jaundice. Positive results, however, should not be relied upon to rule out the possibility of obstructive jaundice. The thymol turbidity is frequently positive if obstruction is complicated by cholangitis.

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ETHYLENE GLYCOL POISONING

A Clinical and Pathologic Study of Three Cases

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CONFUSION in the layman's mind over the varying composition of antifreeze solutions has led to the accidental poisoning of an increasing number of individuals. Under these circumstances ethylene glycol solutions (for example, Prestone) have been ingested with serious results in each instance. Such accidents might be avoided to some extent if containers of ethylene glycol solutions were more conspicuously labeled Poison.

Ethylene glycol ($HOCH_2CH_2OH$) is a colorless, odorless liquid which has a characteristic pleasant bittersweet flavor. In vivo, ethylene glycol is oxidized to oxalic acid and then to glycollic acid. Its toxicity has been studied rather extensively experimentally by a number of workers.

Experimental Data.—Page[1] studied the effects of ethylene glycol in dogs, rabbits, and rats, and in fatal poisoning he found hemolysis of blood, bloody urine, and distended urinary bladders. Comparing the toxicities of ethylene glycol, propylene glycol, and diethylene glycol, Holck[2] found that animals died twice as fast with ethylene glycol. Laug and co-workers[3] describe weakness, lack of coordination, coma, and death in animals fed ethylene glycol. Pathologic studies revealed hydropic degeneration of convoluted tubules and focal necrosis of the liver. In addition, pulmonary congestion and hemorrhage were noted as well as hemorrhages in the stomach. They found the LD_{50} of ethylene glycol to be about half that of diethylene glycol in most animals. These results were confirmed by Smyth, Seaton, and Fischer.[4]

Kesten and co-workers[5] described experimental renal lesions with calcium oxalate deposits and high blood nonprotein nitrogen values following ethylene glycol administration. Similar lesions were observed by Morris and associates[6] in rats, including the formation of calcium oxalate bladder stones.

Wiley and co-workers[7] recognized the conversion of ethylene glycol to oxalic acid in experimental animals but did not feel that such conversion occurred in amounts sufficient to explain its toxicity. Similar data are presented by Mulinos and co-workers[8] who found crystalline deposits only in chronic toxicity experiments; administration of sodium oxalate in appropriate amounts did not cause death in experimental animals. They found no hydropic degeneration of kidney tubules and felt that animals died of extrarenal causes. Wiley[7] described lymphocytic meningeal reactions in experimental animals following ethylene glycol administration. Newman and associates[9] perfused livers with ethylene glycol and found oxygen consumption depressed and lactic acid formation increased.

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Received for publication, Jan. 14, 1948.

Experimental evidence thus suggests that ethylene glycol acts as a depressant to the central nervous system and produces lesions in the kidney, liver, and lung.

Clinical Data.—Bachem (1917)[10] drank ethylene glycol and observed an increase in excretion of oxalic acid in the urine. In 1927 Page[1] drank 15 c.c. diluted without effect.

In 1930 Hausen[11] reported two cases with recovery. Decapsulation of the kidney was performed in one instance. In the same year Brekke[12] reported two cases successfully treated with unilateral renal decapsulation. Three additional cases have been reported in brief.[13, 14]

In 1943 Boemke[15] reported four fatal cases with necropsy findings. Two of his patients exhibited hyperemia of the brain and leptomeninges, small ring hemorrhages in the brain stem, and perivascular cell infiltrates in the gray and white matter of the cerebrum. Sections of the kidneys revealed dilated tubules with fat droplets in the epithelium. The lumen of tubules contained hyaline material, crystals, and red blood cells. Boemke attributed changes in the brain to previous typhus infections.

A clinical and pathologic report of eighteen fatal cases was published by Pons and Custer.[16] The clinical description of these cases emphasized the predominance of the neurological manifestations, that is, coma, convulsions, and so on. The urine contained oxalate crystals in each recorded instance. All tissues were congested. Calcium oxalate crystals were seen in all kidney sections and in occasional brain sections. The authors emphasized the inflammatory reaction seen perivascularly in the brain and diffusely in the meninges. In some instances hemorrhage accompanied this infiltration. Degenerative changes in cells of the brain were also found. These authors felt the lesions in the central nervous system explained the fatal outcome in acute severe ethylene glycol poisoning.

Another case with clinical and pathologic findings has been reported in detail by Milles.[17] This patient was euphoric and later depressed; he developed hypertension and fever and died twenty-two hours following the ingestion of ethylene glycol. Autopsy revealed pulmonary edema, injection of pial vessels, and swollen and vacuolated liver cells. The glomerular capillaries were distended and tubular epithelium was swollen. Crystals were seen in the tubules. The acute course was attributed to oxalate poisoning, and several therapeutic suggestions were given with this in mind.

CASE PRESENTATIONS

The three patients herein presented were admitted to the station hospital at Los Alamos, N. M., following a drinking bout during which one of the victims induced his friends to share his "wine." The quantities consumed could not be estimated with any accuracy. The material was consumed from three to five hours prior to admission.

CASE 1.—A. B., a 36-year-old Spanish-American, was admitted to the hospital in coma about three hours after he had ingested the "green wine."

Physical Examination: The temperature was 101° F., pulse rate, 128, and respiratory rate, 44. Slight cyanosis was present. Greenish froth exuded from the nose and mouth. The pupils reacted to light. Heart, lungs, and abdomen were negative. Blood pressure was 120/90. Reflexes were absent and extremities were flaccid.

Laboratory Examination: The red blood count was 5,350,000, hemoglobin content, 14.5 Gm. per 100 c.c., and white blood count, 32,300. Differential white cell count was as follows: neutrophiles, 91 per cent, stab forms, 4 per cent, metamyelocytes, 1 per cent, lymphocytes, 1 per cent, monocytes, 3 per cent. Urinalysis showed a specific gravity of 1.009, acid reaction, a trace of albumin, 2 plus sugar (glucose iv), numerous red blood cells, and a few crystals resembling hippuric acid. Spinal fluid was slightly cloudy and contained 214 white blood cells per cubic millimeter; globulin was 4 plus, and total protein was 170 mg. per cent. Blood nonprotein nitrogen was 81 mg. per cent, and carbon dioxide combining power was 7.5 volumes per cent.

Course: The stomach was immediately lavaged with 4 per cent solution of sodium bicarbonate, and 500 c.c. of this solution were left in the stomach. One liter of 10 per cent glucose in distilled water and 1 liter of one-sixth molar sodium lactate in normal saline were given intravenously. Oxygen was given by nasal catheter. The patient remained unconscious; the pulse became very rapid and weak and the respirations rapid, deep, and labored. The nasal tube was inserted twelve hours after admission and 2 Gm. of sodium bicarbonate were given by tube each hour for six doses. A series of generalized convulsions began at this time and the blood pressure fell to 60/20. Magnesium sulfate was given intramuscularly in an effort to control convulsions. An additional 2 liters of one-sixth molar sodium lactate were given. The patient expired twenty hours after admission.

Case 2.—M. S., a 27-year-old Spanish-American, was found unconscious in a furnace room, where he evidently had collapsed while attempting to carry out his duties as a fireman. Approximately three hours previously he was reported to have consumed some green liquid with the first patient.

Physical Examination: Temperature was 98° F., pulse rate, 90, respiratory rate, 32, and blood pressure, 140/90. The patient was comatose but reacted slightly to painful stimuli. Pupils were dilated but reacted to light. Heart, lungs, and abdomen were not notable. The limbs were flaccid and reflexes were absent.

Laboratory Examination: Blood count revealed 5,820,000 red blood cells, 18.8 Gm. hemoglobin, and 37,200 white blood cells. The differential white cell count was as follows: neutrophiles, 85 per cent, stab forms, 4 per cent, lymphocytes, 1 per cent, monocytes, 10 per cent. Urinalysis showed a specific gravity of 1.009, acid reaction, a trace of albumin and sugar, 3 to 5 red blood cells per high-power field, and numerous crystals resembling hippuric acid. The spinal fluid was slightly cloudy and contained 62 white blood cells per cubic millimeter; globulin was 4 plus and total protein, 170 mg. per cent. Blood nonprotein nitrogen was 66 mg. per cent, and carbon dioxide combining power, 7.5 volumes per cent.

Course: Therapeutic efforts were identical with those described in Case 1, namely gastric lavage, liberal administration of fluids, alkali, and oxygen. The patient remained comatose; the pulse rate rose to 136 and the respiratory rate to 36. Breathing was deep, labored, and rapid. Blood pressure rose to 190/105. Generalized convulsions began fifteen hours after admission, following which the patient failed rapidly with a fall in blood pressure to 70/40. The patient expired seventeen hours after admission.

Case 3.—P. B., a 53-year-old Spanish-American, was brought to the hospital for observation because he had consumed one drink of the same liquid. The patient was at work when contacted and except for appearing slightly intoxicated and unsteady was in satisfactory condition. In addition to the drink, which was consumed eleven hours before admission, the patient had had several bottles of beer and several "shots" of whiskey during the day. There had been no nausea, vomiting, or other untoward symptoms.

Physical Examination: The rectal temperature was 98° F., pulse rate, 130, and respiratory rate, 24. The patient was well oriented, cooperative, and did not appear ill. Conjunctival vessels were injected. The pupils were small and reaction to light was questionable. No other abnormalities were noted.

Laboratory Examination: Red blood cell count was 4,980,000, hemoglobin content, 12.4 Gm. per 100 c.c., white blood cell count, 40,000. Differential white cell count revealed neutrophiles, 89 per cent, stab forms, 10 per cent, lymphocytes, 3 per cent, and monocytes, 8 per cent. Urinalysis showed a specific gravity of 1.009, acid reaction, no albumin or sugar; there were numerous red blood cells and crystals resembling hippuric acid. Stomach content showed no free hydrochloric acid and 4 plus occult blood. Spinal fluid contained 59 white blood cells per cubic millimeter, 4 plus globulin, and total proteins of 124 mg. per cent. Blood nonprotein nitrogen was 59 mg. per cent and carbon dioxide combining power, 5 volumes per cent.

Course: Three hours after admission the patient became restless and lapsed into coma. Oxygen was administered and 1 liter of one-sixth molar sodium lactate was given intravenously. Respirations were shallow and rapid. Blood pressure was 195/80. Nine hours after admission respirations became very labored and shortly thereafter the patient became cyanotic and respirations ceased. The patient was revived with artificial respiration, adrenalin, and Coramine, but remained comatose. The pulse remained rapid and weak and respirations were deep and labored. A second liter of one-sixth molar sodium lactate was given. Sodium bicarbonate (2 Gm.) was given by nasal tube every two hours for four doses. The patient expired seventeen hours after admission.

Autopsy Findings.—

Gross Pathology: The external appearance in all three instances was not remarkable. Livor mortis and a moderate degree of rigor mortis were present. Following the primary incision, in each case the urinary bladder was found to be greatly distended to the level of the umbilicus and filled with clear, pale urine. In all three, the gross appearance of the organs was nearly identical save for the presence of active tuberculous pulmonary lesions in Cases 1 and 2.

All organs showed moderate to severe engorgement of vessels. Although the peritoneal surfaces were not remarkable, the pleural surfaces, both visceral and parietal, were studded with petechial hemorrhages, hemorrhagic streaks, and several large subpleural hematomas. The lungs were markedly congested, more so in the posterior dependent portions, and on cross section revealed multiple small hemorrhagic areas scattered throughout the parenchyma, varying in number in each case. The pericardium was of normal appearance, but the epicardial surface was streaked with large, recent hemorrhages sometimes reaching the proportions of hematomas. These hemorrhages were found principally along the coronary vessels and their branches and over the auricles. In one case tiny hemorrhages were observed in the adventitia of the superior vena cava, near the heart; in another, similar petechial hemorrhages were seen in the adventitia of the ascending aorta. The relative size of the hearts was not remarkable and no cardiac dilatation was noted. Grossly the myocardium was not significantly altered, but in one case small hemorrhages were seen in the endocardium.

The stomachs and intestines were not remarkable, except for congestion and occasional small mucosal hemorrhages. The mucosa of the esophagus, in each case, was smoky-gray in color with one or two small points of epithelial sloughing. The livers were not enlarged; they were deep reddish brown in color with

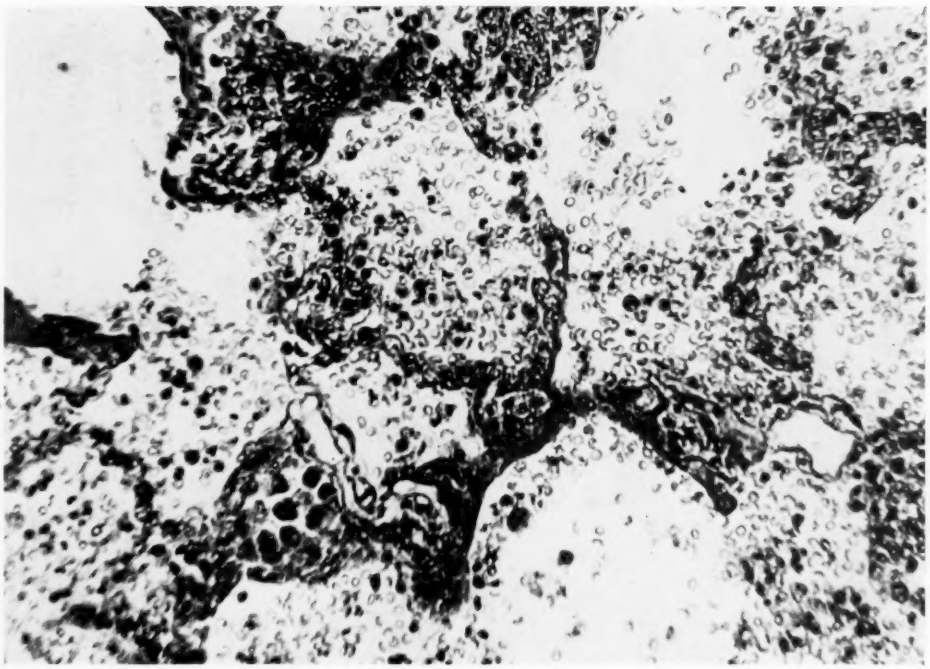

Fig. 1.—Section of lung through hemorrhagic area showing deposition of fibrin-like material lining the alveolar walls and extensive extravasation of red cells into the alveolar spaces (×100).

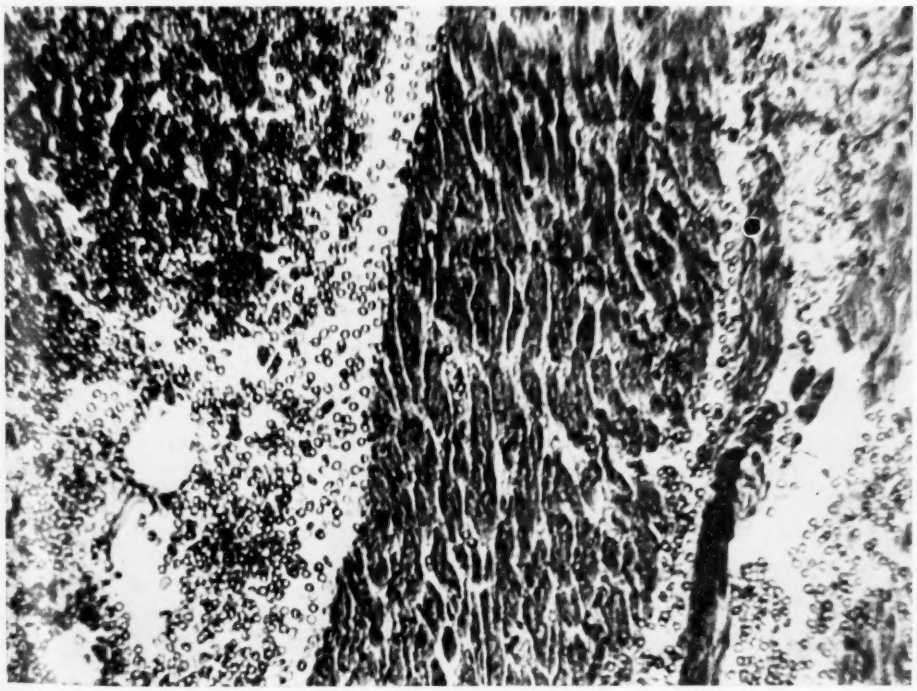

Fig. 2.—Section of superficial myocardium showing extensive recent extravasation of blood (×100).

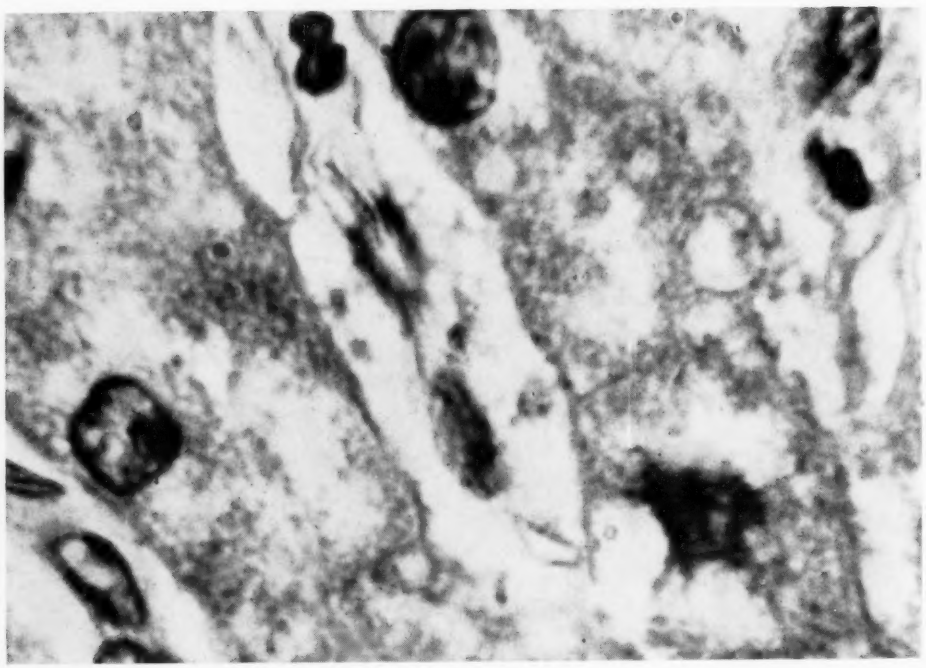

Fig. 3.—Liver showing marked perisinusoidal edema containing albuminous deposits (×970).

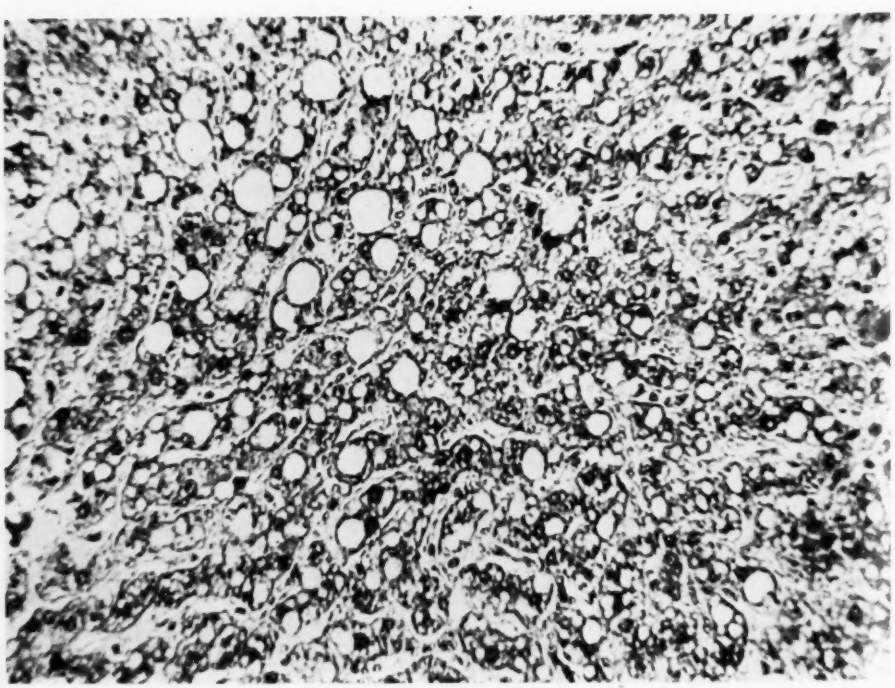

Fig. 4.—Liver showing marked fatty infiltration (×100).

distinct markings, but showed no other significant changes. The kidneys were of normal size and markedly congested, and in one a few petechial hemorrhages were noted under the capsule. Small hemorrhages were seen in the pelvic lining of all and in the bladder mucosa of two.

The brain, in each case, showed marked vascular engorgement with scattered petechial hemorrhages over the surfaces and within the sulci. The cerebrospinal fluid was grossly clear and the ventricles were not dilated. Coronal sections at 1.0 cm. intervals showed only congested and dilated vessels in two instances, and in a third, a few scattered petechial hemorrhages in the white matter. In two instances, recent hemorrhage was present in the mastoid cells.

Histopathology: The microscopic pictures of all three were relatively similar to one another except for variations in degree which will be mentioned. No space will be given to description of the tuberculous lesions of the lungs. Tissue sections from the lungs revealed moderate alveolar distension, emphysema, and variable degrees of capillary congestion, especially in the dependent portions of the lungs. In all lobes, many small and large areas of recent hemorrhage within the parenchyma and subpleurally were noted. The alveolar walls intermittently presented a uniform pink-staining material having a fibrinoid appearance (Fig. 1). This material formed heavy deposits lining the alveolar walls, sometimes sloughing off into the alveolar spaces. Some of the capillary endothelial cells were swollen and in many places actually appeared degenerate and became contiguous with the fibrin-like deposits. In Cases 2 and 3 congestion and hemorrhage were marked, but the fibrin-like deposits were seen only in scattered places.

Sections of the myocardium, in each case, showed many large recent extravasations of red cells under the epicardial membrane and scattered throughout the myocardium between the muscle fiber bundles (Fig. 2). The vessels, again, were markedly dilated, and very occasionally hyalin-like degeneration of the capillary wall, similar in character to that of the pulmonary capillaries, was found. There were areas in which degenerative changes of the myocardial fibers had taken place consisting of interstitial edema, swelling of the fibers, and loss of cross striations. A fat stain (sudan II) revealed a diffuse deposit of finely granular lipoid substance in the muscle fibers.

Although grossly the livers showed no specific changes, the sections evinced a fairly well-preserved architecture with the presence of wide zones of edema between the liver cord cells and the venous sinusoids. In most instances, these wide edematous spaces contained variable quantities of amorphous albuminous material (Fig. 3). The liver cells, although well preserved, were filled with many large vacuoles. Sudan II staining revealed numerous fat droplets in the cytoplasm of the liver cells. This was especially striking centrally, but affected about three-fourths of the lobule (Fig. 4). Sections of the spleen showed foci of hemorrhage and diffuse congestion.

Sections of the esophagus revealed rather well-preserved epithelium which was raised from its base and interrupted in two or three places. An occasional nucleus appeared pyknotic but, for the most part, the cells were normal and stained well. The immediate subepithelial layers were intensely congested with

Fig. 5.—Tufted crystalline deposits in renal tubule. Note the well-preserved epithelial lining (×970).

a few small hemorrhages. The vessels, however, did not show any evidence of degeneration of focal necrosis. Sections of the stomach and small and large intestine were not especially remarkable. There was marked capillary dilatation, engorgement, and edema of the lamina propria. Occasionally small hemorrhages were seen.

The kidneys showed the most striking distention of capillaries, arterioles, and venules alike. In many instances, there were small amounts of blood extravasated outside the vessel walls. The glomeruli were large but without proliferation of epithelium or endothelium; here, too, there was marked distention of the capillary loops forming lakes of blood which could be interpreted as true stasis. There was no evidence of capillary degeneration nor of hyalin-necrotic mural changes such as were seen in the lung. Although the lining epithelium was well preserved, approximately two-thirds of the renal tubules contained large grayish crystals, many appearing as shocks of wheat with a central binding similar to sulfathiazole crystals but smaller (Fig. 5); others showed irregular plate-like appearance similar to uric acid crystals. Positive identification of these crystals was not made.

Numerous sections were taken from various portions of the brain (the frontal and occipital lobes, the internal capsule, cerebellum and dentate nucleus, pons, medulla, and olive) and spinal cord. The changes in nearly all

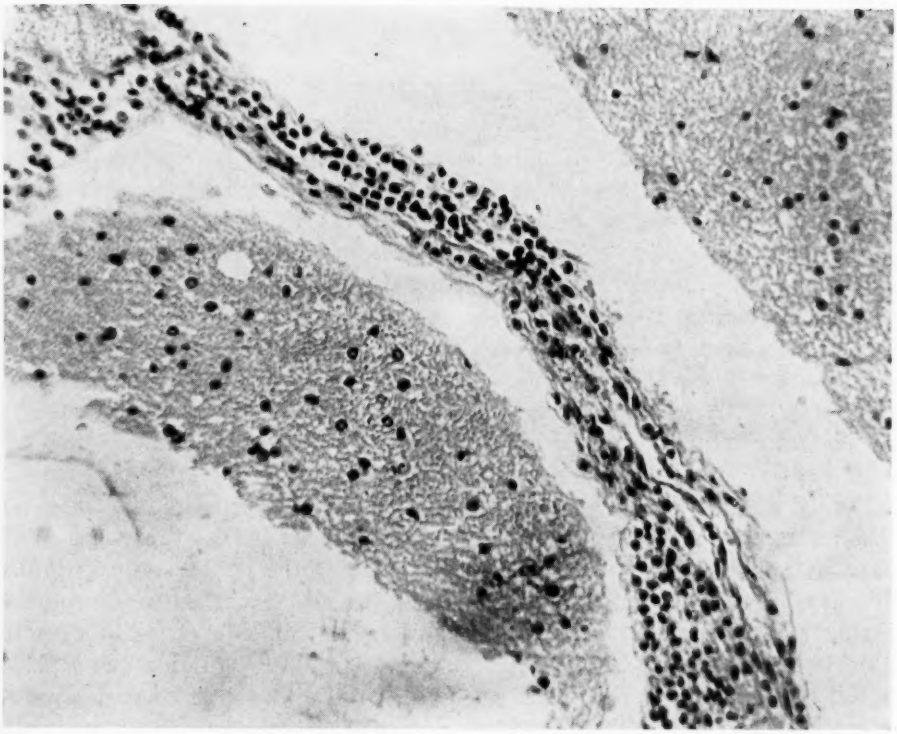

Fig. 6.—Meninges. The thickening of the pia-arachnoid is chiefly perivascular, consisting of predominantly neutrophilic leucocytes, moderate numbers of lymphocytes and pale monocytes, and extravasated red cells (×100).

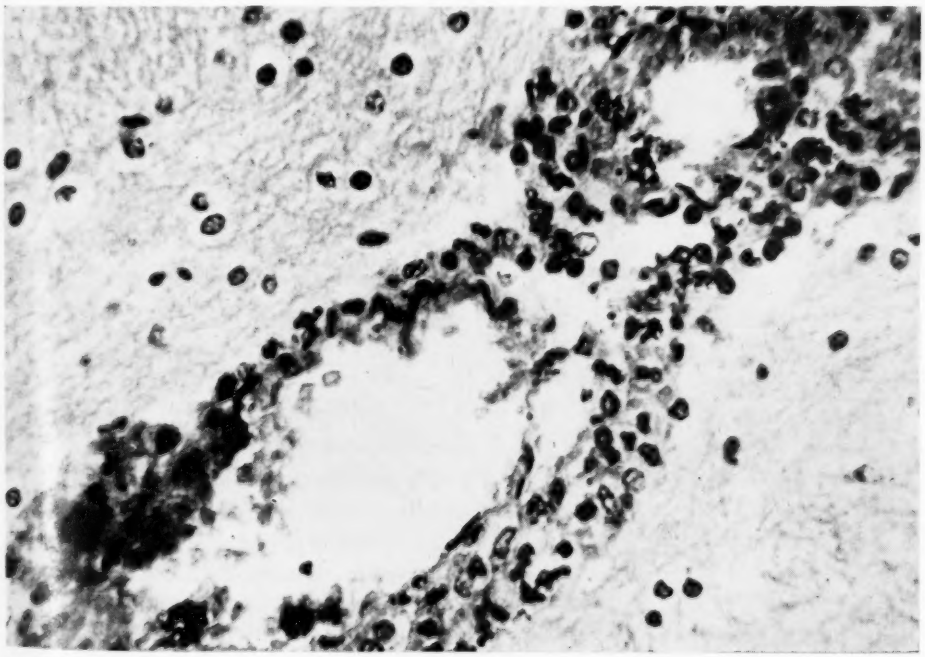

Fig. 7.—Small cerebral vessels showing perivascular edema and intensive diapedesis of leucocytes and red cells (×430).

sections included simple vascular congestion, the diapedesis of leucocytes into the perivascular spaces, the extravasation of red cells, and frank exudative menigo-encephalitis (Fig. 6). Some vessels showed only moderate numbers of leucocytes infiltrating through the vessel walls and filling the perivascular space. This infiltrate consisted chiefly of neutrophiles with varying numbers of lymphocytes and palely stained mononuclear cells. Other vessels showed perivascular infiltrations of equal numbers of leucocytes and red cells (Fig. 7). Occasionally, however, only red cells were seen about the vessels, sometimes in ring form. In only one of these cases (Case 1) was the pia-arachnoid found to be universally infiltrated with extravasated blood, serum, and leucocytes, principally neutrophiles. In this case, the superficial vessels of the parenchyma also showed a marked perivascular cuffing with neutrophiles, lymphocytes, and occasional monocytes. In the remaining two cases only few focal areas of meningeal infiltration were seen, the principal changes being congestion, edema, and focal hemorrhages. These vascular changes were found in all sections of the brain stem and in the spinal cord as well. Some of the small vessels were filled with hemolyzed blood cells. Curiously enough, in one section of the internal capsule (Case 2) several of the small capillaries exhibited crystalline tufts exactly similar to those found in the renal tubules. With toluidine blue stain, in most instances, the ganglion cells appeared to be well preserved and arranged perpendicularly to the brain surface. However, in each case there were scattered focal areas of derangement of these large cells which stained deeply and appeared somewhat pyknotic. Occasionally chromatolysis as well as satellitosis with oligodendroglia was observed. Early chromatolytic changes of the ganglion cells of the spinal cord were also seen. Myelin stains showed no abnormality, and modified Spielmeyer's stain of peripheral nerves showed no evidence of demyelinization of nerve fibers.

Sections of thoracic and abdominal lymph nodes, aorta, bone marrow, prostate, testis, adrenals, thyroid, pituitary, and peripheral nerves showed no significant alterations.

TABLE I. ETHYLENE-GLYCOL CONTENT OF TISSUES AND URINE
(EXPRESSED IN MILLIGRAMS PER CENT)

	CASE 1	CASE 2	CASE 3
Brain	500	450	400
Liver	133	100	662
Kidney	410	440	500
Urine	1650	1000	1000

Chemical Laboratory Analysis: Portions of brain, liver, and kidney were analyzed for presence or absence of common poisons and ethylene glycol. Equal samples in each case were submitted as well as samples of the urine and gastric contents. The post-mortem gastric contents were negative. The tissue sections were entirely negative for the common volatile poisons, alkaloids, and heavy metals. Special procedures were instituted for the qualitative and quantitative assay of ethylene glycol and the results are incorporated in Table I.

Technique employed for detection of ethylene glycol in body tissues and urine.

Brain, Liver, and Kidney: Twenty-five grams of tissuue were chopped finely with scissors and transferred to a 500 ml. side arm distilling flask; 20 to 25 c.c. distilled water were added. Steam distillation was carried out until 50 c.c. of distillate were obtained. The residue was contrifuged and the total volume measured and made up to 50 cubic centimeters. Two cubic centimeters of 2/3 N sulfuric acid and 2 c.c. 10 per cent sodium tungstate were added to 16 c.c. of the supernatant fluid. To 5 c.c. of the filtrate were added 2.5 c.c. 2 per cent potassium permanganate and 0.6 c.c. concentrated sulfuric acid. After standing five minutes, 0.5 c.c. 10 per cent oxalic acid and 0.6 c.c. concentrated sulfuric acid were added. After the solutions became colorless, 5 c.c. Schiff's reagent were added. After fifteen minutes the unknowns were compared with standards that were prepared simultaneously. Normal body tissues were employed as controls. These were uniformly negative for ethylene glycol.

Urine: Urine was tested in a similar manner. One cubic centimeter urine plus 4 c.c. distilled water were used instead of the 5 c.c. filtrate employed in tissue analysis. Normal controls were again negative.

Formaldehyde was detected in urine by adding 1 c.c. Schiff's reagent to 3 c.c. distilled water and 1 c.c. urine. The pink colors were compared with standards prepared simultaneously.

The urine also was found to contain small quantities of formaldehyde (presumably an oxidation by-product) in the following amounts:

Case 1	4.0 mg. per cent
Case 2	5.2 mg. per cent
Case 3	4.4 mg. per cent

The heaviest concentrations of the specific agent in the brain and in the urine were found in Case 1. Correspondingly, the tissue sections showed the most dominant lesions histologically.

DISCUSSION

The bottle from which these patients drank an unknown quantity was shown by analysis to contain a 40 per cent solution of ethylene glycol. Tests for methyl alcohol on the same liquid were negative.

The picture presented by all three patients was strikingly similar, being characterized by coma, acidosis, hypertension, convulsions, and death. The widespread toxic reaction was evidenced clinically by the extreme leucocytosis, albuminuria, microscopic hematuria, nitrogen retention, lowered carbon dioxide combining power and spinal fluid changes. The therapeutic efforts had no apparent benefical effect.

The pathogenesis of ethylene glycol toxicity in man is not clearly understood. Pons and Custer[16] attributed the main effect to a chemical meningitis, and Milles,[17] to oxalate poisoning. The evidence presented in these three cases seems to indicate widespread capillary damage. This is assumed to be the primary lesion. Widespread hemorrhages result, as seen in pleura, lung, heart, pericardium, kidney, and brain. In all three, the brains and vessels were congested with perivascular infiltration of leucocytes and red blood cells. In Case 1 the changes in the meninges and brain were more diffuse and well-developed, suggesting a chemical meningo-encephalitis. The edema and fat infiltration seen in the liver were striking and presumably represent a direct toxic effect. Crystals (presumably calcium oxalate) seen in kidney tubules and in the brain were considered a characteristic but unimportant finding.

The demonstration of ethylene glycol in body tissues and in post-mortem urine serves to demonstrate its wide distribution corresponding with the diffuse pathologic lesions. The high concentration of the chemical in the urine re-emphasizes that the principal path of excretion is via the kidney.

Specific therapeutic measures do not seem to be at hand.

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THE ACTION OF PILOCARPINE ON THE LUNGS IN NORMAL AND ASTHMATIC SUBJECTS

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THE reaction of the tracheobronchial tree to histamine and acetyl-beta-methylcholine (Mecholyl chloride) in normal and asthmatic subjects has been described by one of us (J. J. C.) in previous reports.[1-3] While it was shown that in the latter subjects the parenteral injection of either histamine or Mecholyl chloride induced asthma-like attacks, with reduction in the vital capacity, it appeared that the respiratory tract was more reactive to Mecholyl chloride than to histamine in many of the cases. In view of the implication of these findings with regard to the etiology and possibly the therapy of spontaneous asthma, it was felt that further study of the role of the parasympathetic nervous system in bronchial asthma was warranted. The present communication deals with the action of a parasympathomimetic drug, pilocarpine, on the tracheobronchial tree in normal and asthmatic subjects. Comparison of the respiratory reaction after pilocarpine with that following Mecholyl chloride, and in some instances Prostigmine, is also made in an attempt to furnish further information on the pharmacodynamic action of pilocarpine.

Few studies on pilocarpine have been made in man except those dealing with the action of the drug on the sweat glands. This substance acts on cells innervated by postganglionic cholinergic fibers and exhibits most of the muscarinic but not the nicotinic properties of acetylcholine.[4] It is not clear whether or not pilocarpine produces a discharge of epinephrine from the adrenal gland for there are conflicting reports.[5-10] The occurrence of such a discharge would influence our studies since epinephrine causes pronounced bronchodilation and counteracts the action of some parasympathomimetic drugs on the respiratory tract in asthmatic subjects.[11] In 1921 Alexander and Paddock[12] gave 3 mg. of pilocarpine subcutaneously to twenty asthmatic patients, and in ten, asthmatic-like attacks were precipitated as a result of the injection.

METHOD AND MATERIALS

Pilocarpine hydrochloride was administered intramuscularly or intravenously in doses of 1 to 5 mg. to a group of normal subjects and to a group of asthmatic patients. The reaction of the respiratory tract was measured chiefly by recording the vital capacity on a rapidly moving drum according to a method previously described.[1] The first group was composed of ten subjects who had no personal or family history of allergy and no signs or symptoms of allergic disease or bronchitis. In the second group were seventeen ambulatory patients with bronchial asthma. No attempt was made to classify the type of asthma, but the majority of patients were young adults subject to attacks at any time during the year. For

This work was supported in part by a grant from the Upjohn Company, Kalamazoo, Mich.
From the Robert Dawson Evans Memorial, Massachusetts Memorial Hospitals, and the Department of Medicine, Boston University School of Medicine.
Received for publication, Jan. 26, 1948.

purposes of comparison they were divided into three groups, depending chiefly on the number and severity of the attacks. The majority of these subjects had been studied previously, had performed numerous vital capacity tests, and were well trained and very cooperative.

Pilocarpine hydrochloride* was dissolved in sterile isotonic saline to a concentration of 10 mg. per milliliter and placed in a sterile rubber-capped bottle. The solution was replaced at monthly intervals and stored in a refrigerator when not in use.

RESULTS AND COMMENT

Normal Subjects.—In the group of ten normal subjects the intravenous injection of 2 to 5 mg. of pilocarpine produced no significant reduction in the vital capacity (Table I). Similar results were obtained after 6 mg. doses of Mecholyl chloride given intramuscularly in a previous study.[3] There was no subjective sensation of tightness in the chest after pilocarpine, however, as

TABLE I. EFFECT OF THE INTRAVENOUS ADMINISTRATION OF 2 TO 5 MG. OF PILOCARPINE HYDROCHLORIDE ON THE VITAL CAPACITY IN TEN NORMAL SUBJECTS

SUBJECT	AGE (YR.)	SEX	PILOCARPINE (I.V., MG.)	VITAL CAPACITY	
				BEFORE DRUG (Ml.)	CHANGE (%)
T. H.	32	M	2	4441	- 4
			2	4107	-10
R. C.	29	M	3	3459	+ 5
L. J.	25	M	3	5214	- 1
H. O.	23	M	2	4953	+ 5
S. L.	30	M	2	4577	+ 0.2
			5	4452	- 0.4
J. C.	32	M	2	4201	+ 2
			5	4107	+ 4
S. K.	30	M	3	4389	- 0.5
			5	4452	- 4
J. S.	26	M	3	3563	+12
R. F.	25	M	3	4765	- 0.2
P. K.	32	M	3	4065	+ 1

noted following the administration of Mecholyl chloride. Coughing was experienced occasionally and salivation persisted for a longer period of time after pilocarpine than after Mecholyl chloride. The increase in vital capacity after pilocarpine in some of the subjects perhaps might have been due to the fact that they were not so well trained as the asthmatic group. However, the control vital capacities were uniform. The sweating response in this group following comparable doses of pilocarpine was apparently uniform as judged by clinical observation. The heart rate was consistently elevated. In these respects the findings were similar to those obtained with Mecholyl.

Asthmatic Subjects.—All of the seventeen asthmatic patients suffered a reduction in vital capacity after the administration of 1 to 4 mg. of pilocarpine intramuscularly or intravenously and in many instances the reaction was severe (Table II). However, one subject (J. B.), who had mild asthma, had a reduction of only 2 per cent with 4 mg. by vein, and another subject (R. B.), who had

*Supplied by Merck and Company, Inc., Rahway, N. J.

TABLE II. EFFECT OF THE INTRAMUSCULAR OR INTRAVENOUS ADMINISTRATION OF 1 TO 4 MG. OF PILOCARPINE ON THE VITAL CAPACITY IN SEVENTEEN PATIENTS WITH ASTHMA COMPARED WITH THE EFFECT OF MECHOLYL CHLORIDE

SUBJECT	AGE (YR.)	SEX	SEVERITY OF ASTHMA			DRUG ADMINISTERED			VITAL CAPACITY	
						PILOCARPINE		MECHOLYL CHLORIDE	BEFORE DRUG	CHANGE
			1+	2+	3+	I.M. (MG.)	I.V.	(I.M. MG.)	(ML.)	(%)
F. G.	29	M			x	3			3469	-16
							1		3647	-17
								4	4159	-61
R. C.	42	F			x		1		2477	-27
W. J.	14	M			x		1		2456	- 8
								2	2278	-37
L. S.	23	F		x			2		2707	-22
								4	2257	-30
J. B.	15	M	x				4		3250	- 2
								4	3156	-29
R. B.	25	F			x		2		2424	- 1
								2	2404	-48
R. B.	19	M		x			2		4734	-22
								2	2989	-47
M. M.	38	F			x		1		3093	- 5
								4	3396	-18
H. H.	49	F			x		1		2414	-13
								1	2247	-21
B. S.	13	F			x		2		1996	-81
								2	1954	-69
W. N.	23	F			x		2		2738	-29
								2	2613	-77
A. L.	33	M			x		2		3584	-12
								4	4504	-45
H. W.	31	F			x		2		2801	-46
								4	2424	-70
W. F.	27	M	x				2		5225	- 5
								6	4681	-31
J. D.	16	F			x		2		2957	- 9
								1	3103	-46
F. W.	45	F			x	3			2853	-10
							4		2560	-35
								2	2529	-36
W. B.	47	M			x	3			3145	-16
							3		3010	-33
								2	2748	-28

1+, Asthma patients with only a past history of asthma or with one or two attacks a year; 2+, asthma patients with asthmatic paroxysms one or two times a month; 3+, asthma patients with one or two attacks or more a week.

daily asthmatic paroxysms, had a reduction in vital capacity of only 1 per cent with an intravenous dose of 2 milligrams. The latter subject also experienced a reduction in vital capacity of only 3 per cent after histamine, but in contrast, the intramuscular administration of 2 mg. of Mecholyl chloride was followed by a decrease in vital capacity of 48 per cent from the resting level of 2,404 milliliters. All the asthmatic patients given up to 6 mg. of Mecholyl chloride in a previous study also reacted with a reduction in vital capacity. The intensity of the asthma-like attack and the degree of reduction in vital capacity were greater after Mecholyl chloride than after pilocarpine with the doses used in these studies.

The degree of flushing, salivation, and sweating in the asthmatic patient did not appear to differ from the responses seen in the normal subjects after comparable doses of pilocarpine. In this respect our findings differed from those of Alexander and Paddock,[12] who concluded that flushing, salivation, and sweating were somewhat more pronounced in asthmatic patients than in normal subjects with similar doses of pilocarpine. The reaction pattern of the tracheobronchial tree, as determined by repeated vital capacity tests at intervals after the injection of pilocarpine, was variable, in contrast to the reaction after histamine and Mecholyl.

Intramuscular Injection.—After the intramuscular injection of 3 to 4 mg. of pilocarpine in the asthmatic patients, flushing, salivation, and mild sweating appeared within a few minutes, but the maximum reduction in vital capacity did not occur for from six to twenty-five minutes. The following case illustrates the reaction of Subject F. G. (Fig. 1) to the intramuscular injection of 3 mg.

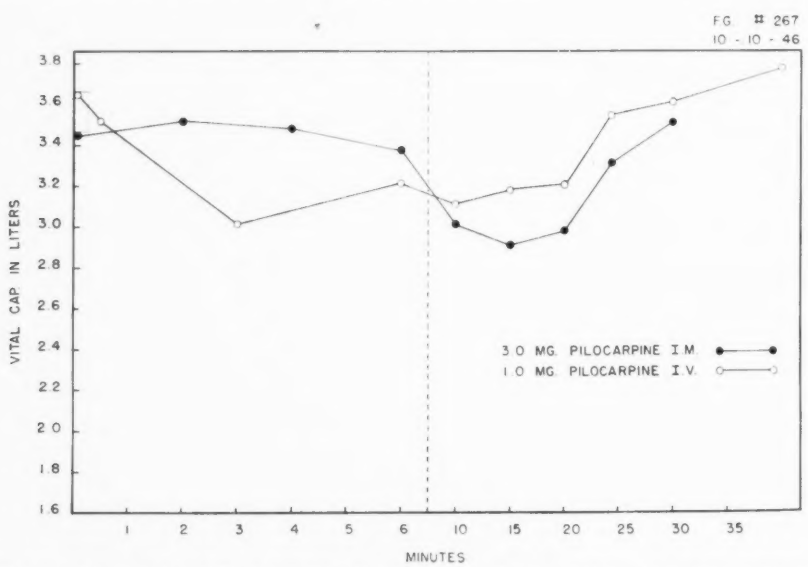

Fig. 1.—Comparative effect on the vital capacity of pilocarpine given intravenously and intramuscularly in Subject F. G. Horizontal line represents time in minutes after the injection. Interrupted vertical line indicates change from one- to five-minute time intervals. The unshaded box on the left shows the control range of vital capacity determinations.

of pilocarpine. In the first six minutes after the injection, despite the subjective sensation of tightness in the chest, there was no reduction in the vital capacity. However, fifteen minutes following the pilocarpine administration there was a reduction of 543 ml. in the vital capacity and wheezing appeared. The reaction to the drug had disappeared in thirty minutes and the vital capacity was near the resting level. The delay in appearance of tightness in the chest and the more prolonged delay in reduction in the vital capacity following the injection of pilocarpine clearly is not due to slow absorption, since other systemic effects such as salivation and flushing are manifest within a few minutes after administration of the drug. In this respect the reaction of the

tracheobronchial tree after intramuscular pilocarpine differs from the reaction due to intramuscular Mecholyl chloride. After the latter, a notable reduction in vital capacity is present in two minutes and the maximal effect is present in four to six minutes. In many cases the vital capacity may have returned nearly to the resting level fifteen minutes after the intramuscular administration of the drug. It is interesting to note that the reduction in vital capacity in asthmatic subjects due to intramuscular or intravenous Prostigmine methylsulfate[13] is similarly delayed in onset and resembles the tracheobronchial reaction due to intramuscular pilocarpine. As will be shown later, a second and delayed reduction in vital capacity was also found in many of our subjects after the intravenous administration of pilocarpine. The precise explanation for this delay of tracheobronchial reaction to pilocarpine is not clear. It is possible that the liberation of epinephrine as a result of the injection may in some way manifest a temporary protecting action against the tracheobronchial effect of the drug. It will be shown subsequently that epinephrine may afford protection against the effect of pilocarpine in the lungs without affecting the flushing, salivation, and sweating that result from its administration. Further studies are necessary to explain these findings.

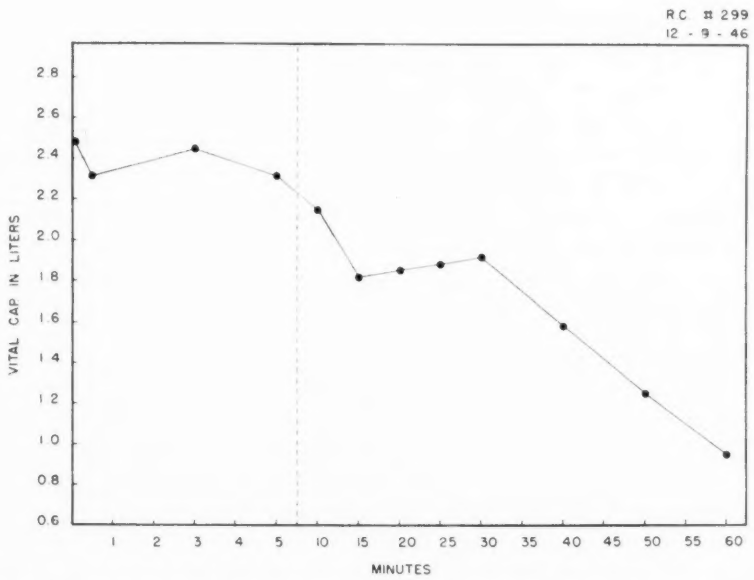

Fig. 2.—Changes in the vital capacity during the precipitation of an asthmatic attack in Subject R. C. with 1.0 mg. of pilocarpine given intravenously. Horizontal line represents time in minutes after the injection. Interrupted vertical line indicates change from one- to five-minute time intervals.

Intravenous Injection.—After the intravenous administration of 1 to 4 mg. of pilocarpine the greatest reduction in vital capacity in most of the patients with asthma occurred in thirty seconds. In a few cases there was a greater reduction three minutes after the administration of the drug. In many cases, after a well-established trend in the vital capacity toward the resting levels,

there was an evanescent secondary decrease in vital capacity ten to fifteen minutes after injection. This second reduction, although small, appeared to be a result of the pilocarpine injection since the subjects were well trained and such variations were not ordinarily experienced after histamine and mecholyl. The decrease in vital capacity varied in amount and was never of the same degree as the initial response to the drug. Even in the same subject it varied with successive identical doses of pilocarpine and did not always occur.

In one patient (R. C.) (Fig. 2) a dose of 1 mg. of pilocarpine produced a notable reduction in vital capacity, and when a definite trend toward normal, together with subjective improvement, had been established, an asthmatic attack occurred. A marked, further decrease in vital capacity resulted. This attack was readily alleviated by inhalation of aerosolized Isuprel 1-(3′, 4′-dihydroxyphenyl)-2-isopropylaminoethanol hydrochloride.*

The decrease in vital capacity following the intravenous injection of pilocarpine was greater than that due to intramuscular administration of an equal dose of the drug, but the ratio was variable. In Subject F. W., 3 mg. of pilocarpine injected intramuscularly produced a reduction in vital capacity of 9 per cent, or 282 ml., whereas an identical dose given intravenously caused a decrease of 18 per cent, or 501 milliliters. On another occasion 4 mg. of pilocarpine were given intramuscularly and the vital capacity decreased 8 per cent, or 198 ml., whereas a similar dose by vein caused a reduction of 35 per cent, or 909 milliliters. In another subject (W. B.) 3 mg. of pilocarpine injected intramuscularly reduced the vital capacity 9 per cent, or 292 ml., whereas the same dose given intravenously caused a decrease of 33 per cent, or 1,004 milliliters. In comparison with these studies the reaction of the respiratory tract to Mecholyl chloride given intravenously appeared to be ten to fifty times greater than an identical dose given by the intramuscular route.

In four subjects, 0.5 mg. of Prostigmine methylsulfate was administered fifteen to thirty minutes prior to the injection of pilocarpine. In two instances it appeared to augment the action of pilocarpine on the tracheobronchial tree. For example, in Subject J. D. 2 mg. of pilocarpine given intravenously produced a reduction of 291 ml. in the vital capacity from the resting level of 2,957 milliliters. When the level had returned toward normal, 0.5 mg. Prostigmine was injected by vein and the vital capacity fourteen minutes later measured 2,654 ml., a reduction due to Prostigmine itself of 303 milliliters. A dose of 2 mg. of pilocarpine was repeated one minute later and a further reduction of 428 ml. was noted. The systemic effects of pilocarpine—namely, flushing, salivation, and sweating—also appeared to be augmented by the action of Prostigmine. In two instances Prostigmine did not apppear to augment either the tracheobronchial or systemic effects of pilocarpine.

When repeated identical doses of pilocarpine were administered at thirty-minute intervals to five subjects, variable results were obtained. In two subjects similar reductions in vital capacity occured, but in the other three it appeared that with successive doses there was a markedly diminished effect of

*Furnished by Frederick Stearns & Co., Detroit, Mich.

the drug in reducing the vital capacity. For example, in Subject B. S. (Fig. 3) the intravenous injection of 2 mg. of pilocarpine caused a reduction of vital capacity to 376 ml. from a resting level of 1,996 milliliters. A second injection forty-five minutes later produced a reduction to 993 ml., and a third dose thirty minutes later reduced the vital capacity only to 1,379 milliliters. The

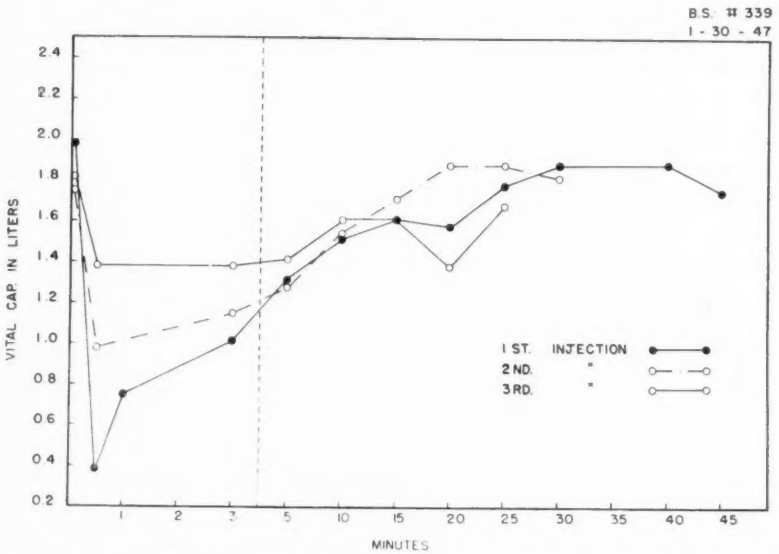

Fig. 3.—Comparative effect on the vital capacity of three 2.0 mg. doses of pilocarpine injected intravenously at intervals. Horizontal line represents time in minutes after the injection. Interrupted vertical line indicates change from one- to five-minute time intervals.

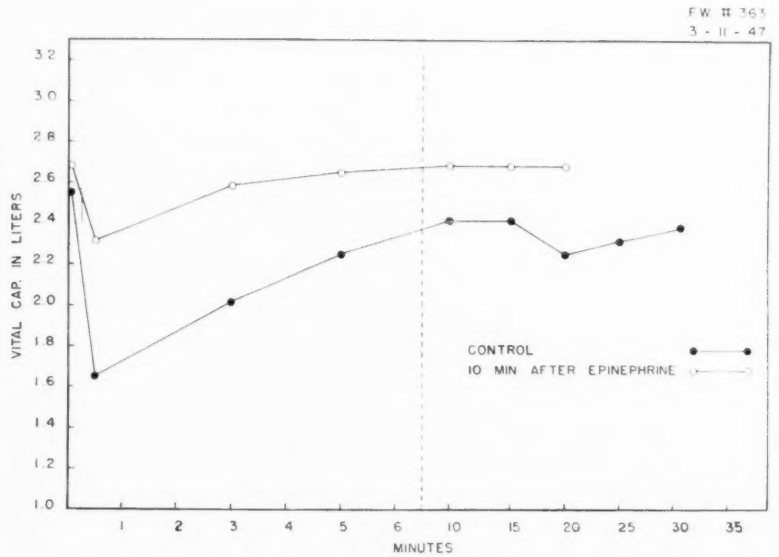

Fig. 4.—The effectiveness of 0.3 mg. epinephrine given intramuscularly in blocking the reduction in vital capacity caused by the intravenous injection of 4.0 mg. of pilocarpine. The horizontal line represents time in minutes after the injection. The interrupted vertical line indicates a change from one- to five-minute time intervals.

subjective sensation of tightness in the chest also became less with repeated injections. In another subject (F. G.) a similar trend resulted after four successive injections at thirty-minute intervals, so that the final injection produced no reduction in vital capacity. When the experiment was repeated several weeks later, however, identical doses of pilocarpine produced similar reductions in vital capacity.

Certain antiasthmatic drugs afforded protection in the reactive subjects against the reduction in vital capacity due to pilocarpine. In two cases epinephrine gave pronounced protection against this reduction. Patient F. W. (Fig. 4), for example, had a resting vital capacity of 2,560 ml., and thirty seconds after the intravenous administration of 4 mg. pilocarpine it measured 1,651 milliliters. After a gradual return toward normal, 0.3 ml. of 1:1,000 epinephrine was injected intramuscularly and five minutes later the vital capacity was 2,686 milliliters. A repeat injection of 4 mg. of pilocarpine produced a reduction in vital capacity to only 2,330 ml., and there was a rapid return to the resting level. It was also noted that although epinephrine appeared to prevent the reduction in vital capacity and the subjective and objective changes in the lungs following the administration of pilocarpine, it did not appreciably affect the salivation, flushing, and perspiration resulting from the injection of the drug. In Patient H. W. epinephrine not only afforded complete protection against the reduction in vital capacity due to pilocarpine but also appeared to reverse the action of the drug. Following the repeat injection of 2 mg. of pilocarpine there was an increase of 460 ml. in the vital capacity, compared with a decrease of 1,098 ml. caused by the same amount of pilocarpine prior to the administration of epinephrine.

Theophylline ethylenediamine likewise prevented the reduction in vital capacity due to pilocarpine. In Subject F. G. the resting vital capacity measured 3,731 ml., and thirty seconds after the intravenous injection of 3 mg. of pilocarpine it measured 2,727 milliliters. When the vital capacity returned to the resting level, 350 mg. of theophylline ethylenediamine were given slowly by vein, and ten minutes later the vital capacity was 3,760 milliliters. A second dose of 3 mg. of pilocarpine then produced a reduction in vital capacity to only 3,647 milliliters. The systemic reaction to pilocarpine was not altered appreciably.

Bellafoline,* a stable preparation of total levorotary alkaloids of belladonna leaves, likewise afforded protection against the reduction in vital capacity due to pilocarpine. Patient R. C., who had previously suffered an acute asthmatic attack following the injection by vein of 1 mg. pilocarpine, was given 0.5 mg. of Bellafoline intramuscularly. Ten minutes later an intramuscular dose of 3.0 mg. of pilocarpine produced a decrease in vital capacity of only 53 milliliters. The degree of protection thus afforded the tracheobronchial tree by epinephrine, theophylline ethylenediamine, and Bellafoline was similar to the protection given by the same drugs against Mecholyl chloride.[11] The other reactions to pilocarpine, such as sweating, flushing and salivation, were only slightly affected by the protecting drugs.

*Furnished by Sandoz Chemical Works, Inc., New York, N. Y.

It appears that attempts to assay the effectiveness of various anticholinergic drugs by their capacity to prevent the asthma-like attacks and the reduction in the vital capacity induced by cholinergic substances would be better carried out with Mecholyl chloride than with pilocarpine as the test substance, since the former drug has a more uniform action. Since anticholinergic agents such as atropine and Bellafoline are very effective in preventing this type of induced asthma it would seem worth while to reinvestigate their use in the treatment of spontaneous asthma, especially since other drugs in this group are available which do not have the unfavorable side reactions of atropine. More support for such an investigation is derived from recent data which indicate that in the asthmatic subject the pulmonary reaction to Mecholyl chloride may be greater and perhaps more important than the reaction to histamine.[3] Confirmation of the pulmonary hyperresponsiveness of asthmatic subjects to cholinergic drugs also suggests that this manner of study should be carried out when surgery on the autonomic nervous supply to the lung is contemplated. It is possible that those subjects who exhibit a more pronounced reactiveness to the cholinergic drugs may benefit most by resection of the posterior pulmonary plexus.

SUMMARY

The reaction of the respiratory tract to doses of 1 to 5 mg. of pilocarpine in a group of ten normal subjects and seventeen patients with asthma was studied chiefly by measurements of the vital capacity. In two of the normal subjects there was a slight decrease in vital capacity after pilocarpine. In contrast, all the asthmatic patients suffered a reduction in vital capacity after the administration of 1 to 4 mg. of pilocarpine intramuscularly or intravenously. After the intramuscular injection of pilocarpine, the period of greatest reduction in vital capacity occurred in six to twenty-five minutes, whereas flushing, salivation, and sweating occurred in a considerably shorter time. The greatest reduction in vital capacity with intravenous pilocarpine usually occurred in thirty seconds, but there was frequently a second reduction ten to fifteen minutes after the injection. Prostigmine appeared to augment the action of pilocarpine in two of four subjects. Epinephrine, theophylline ethylenediamine, and Bellafoline protected the tracheobronchial tree against the reduction in vital capacity due to pilocarpine, but failed to have much effect on the flushing, salivation, and sweating due to the drug.

Clinical implications of the study are discussed.

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A COMPARISON OF THE TOXIC MANIFESTATIONS PRODUCED BY BETA-DIMETHYLAMINOETHYL BENZHYDRYL ETHER HYDROCHLORIDE (BENADRYL) AND TRIPELENNAMINE (PYRIBENZAMINE)

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THE therapeutic value of antihistaminic compounds in many allergic conditions seems now to be well established. Of these compounds, Benadryl (beta-dimethylaminoethyl benzhydryl ether hydrochloride) and Pyribenzamine or tripelennamine (N′-pyridyl-N′-benzyl-N-dimethylethylenediamine) have had by far the most extensive clinical trial. There are a number of other preparations which either in laboratory animals or in man or in both have shown antihistaminic activity equal to or greater than either of these new drugs.[1-3] Nevertheless, until such materials are more thoroughly tested in the human being we must rely mainly upon the two drugs first mentioned whenever an antihistaminic agent is required.

Any comparison of these two substances in human beings is fraught with several difficulties. In the first place, the results in animals cannot be transferred quantitatively, and probably not qualitatively, to human beings, even in regard to the ability of these substances to prevent death from histamine aerosols or anaphylactic shock. Moreover, the alkamine ether and tripelennamine differ in several particulars pharmacologically. The former possesses marked anti-acetylcholine and antibarium chloride activity; the latter has little or none.[1, 3] Benadryl acts oppositely to tripelennamine upon the musculature of the duodenum and the uterus. In the human being both exert some hyoscine-like action, which is more marked with Benadryl.[4, 5]

Comparisons of the therapeutic efficacy of Benadryl and Pyribenzamine are few in number.[3, 6-11] Claims have been made that dose for dose the drugs are equally effective,[6, 7, 10, 11] or that Benadryl[8] or tripelennamine[3, 9] is more powerful. One worker, who found the latter more active than the former,[3] has failed to mention the doses used in the comparisons, although by inference he has left the impression that that employed for Benadryl was often, if not usually, one-half to one-third that of tripelennamine. The second investigator, who more consistently observed better effects in some clinical conditions from tripelennamine, states that the doses of the latter varied from 200 to 400 mg. daily, while those for the alkamine ether were usually 200 mg. daily.[9] Other workers[6-8] made their comparisons on a weight basis.

There is rather general agreement regarding the nature of the side effects following the use of both Benadryl and tripelennamine, but there is marked difference of opinion concerning their incidence and severity. To date, all or

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Received for publication, Nov. 3, 1947.

nearly all of the observations regarding these points have been recorded in the course of treatment of allergic diseases and are therefore subject secondarily to all the influences surrounding the allergic episode. Moreover, there has been no concerted effort to compare the reactions of both drugs in the same subject. Thus the personal equation of the subject has never been eliminated from the final evaluation of the results. In an effort to accomplish these objectives and to afford a sound basis for the comparison of the toxic activity of these antihistaminic compounds over the entire range of therapeutic dosage, the present study was undertaken.

MATERIALS AND METHODS

Ambulatory patients from a general medical clinic of a city hospital were chosen at random except that those with acute illnesses, allergic complaints, or recognizable disturbances of the autonomic nervous system were excluded. The underlying conditions for which these patients sought relief included arthritis, hypertension, arteriosclerosis, and minor upper respiratory, muscular, or digestive complaints. Each subject was further tested for his or her fitness to participate in the investigation by the use of a placebo which resembled the corresponding antihistaminic compound in all its physical characteristics.*

In all, one hundred forty-two subjects, eighty women and sixty-two men ranging in age from 18 to 80 with an average of 44.7 years, completed the tests. Of these, fifty-two were studied while taking Benadryl only, fifty-two while receiving tripelennamine only, and thirty-eight while ingesting successively each of the two antihistaminic compounds. In the last-mentioned subjects a period of fourteen days or more elapsed after one drug had been used before the second was started. Each drug was tried for not less than one week, and any given level of dosage was continued in each subject for not less than one week. A number of subjects were given gradually increasing doses of one or the other drug. The patient continued at each level of dosage for at least one week. Therefore some patients received one or the other of the antihistaminic compounds for periods lasting from four to eight weeks. However, gradually increasing doses of the drug were avoided in the majority of instances, as there was thus produced a tendency to build a tolerance against the unpleasant reactions to the compound.

In order to establish a relatively long-term comparison between the two drugs, each of six subjects was successively followed on gradually increasing doses of first one drug and then the other at daily levels of 150, 300, 450, and 600 mg., respectively. In these subjects each increment in the amount of compound ingested was made after a period of not less than seven days at the immediately preceding level of dosage. Two weeks or more elapsed following the use of one preparation before the other was started.

In Tables I and II the number of patients who developed toxic reactions at each level of dosage and the nature and frequency of various types of reaction

*We wish to express our appreciation to Dr. E. A. Sharp of Parke, Davis & Company, Detroit, Mich., for generous supplies of Benadryl and placebo, and to Dr. R. D. Mayer of Ciba Pharmaceutical Products, Inc., Summit, N. J., for a portion of the tripelennamine.

TABLE I.* THE NATURE AND INCIDENCE OF TOXIC REACTIONS TO BENADRYL AND PYRIBENZAMINE IN RELATION TO DOSAGE OF COMPOUND USED

DAILY DOSE (MG.)	CASES			INCIDENCE AND TYPES OF REACTION											
	TOTAL NUMBER	WITH REACTION		DROWSINESS	DRYNESS OF MOUTH	DIZZINESS	WEAKNESS	NAUSEA AND EPIGASTRIC DISTRESS	VOMITING	CONSTIPATION	SHAKINESS OR JUMPINESS	HEADACHE	ANOREXIA	SPEECH AND VISUAL DISTURBANCES	DIARRHEA
		NUMBER	PERCENTAGE												
Benadryl															
150	14	9	64	8	2	4		1							
300	14	11	78	7	3	2	1						3		
450	13	10	76	7	5	6	2	1		1		1			
600	11	10	91	8	6	4	6	4	1	1	5		1	2	
Total	52	40	77	30	16	16	9	6	1	2	5	1	4	2	
Pyribenzamine															
150	14	9	64	6	5	2		2		1		2	1	1	1
300	14	9	64	5	5	1	1	1				2	1		1
450	15	11	73	7	9	2	1	3		1	1		2		
600	9	6	66	3	6	1	1	2			2	1	2		
Total	52	35	67	21	25	6	3	8	0	2	3	5	6	1	2

*Here are recorded results in 104 patients, one-half of whom received Benadryl and the other half, tripelennamine.

are recorded. It will be noted that at the usually employed daily therapeutic dose, 150 mg., the incidence of side effects was approximately the same for both drugs. The actual percentages are rather high as contrasted with previously reported results. However, since the primary aim was to recognize all unpleasant symptoms rather than to determine therapeutic efficacy, a record was made of each manifestation even though present in minor degree.

TABLE II. THE INCIDENCE OF UNTOWARD EFFECTS IN THIRTY-EIGHT SUBJECTS WHO RECEIVED IDENTICAL COURSES OF TREATMENT WITH BENADRYL AND PYRIBENZAMINE, RESPECTIVELY

NATURE OF MANIFESTATION	NUMBER OF PATIENTS SHOWING SYMPTOMS AT THE LEVELS OF DOSAGE INDICATED									
	11-150 MG.		12-300 MG.		9-450 MG.		6-600 MG.		TOTAL 38	
	B*	P†	B	P	B	P	B	P	B	P
Drowsiness	7	4	5	3	5	3	6	3	23	13
Dryness of mouth	2	5	3	5	3	6	2	5	10	21
Dizziness	3	2	2	1	4	2	2	1	11	6
Gastrointestinal disturbances	1	3	0	1	2	2	3	1	6	7
Headaches	0	2	0	1	0	0	0	1	0	4
Anorexia	0	1	2	2	0	2	0	1	2	6
Palpitation	0	0	0	1	0	0	0	0	0	1
Tinnitus aurium	0	1	0	0	0	0	0	0	0	0
Weakness	0	0	0	1	1	1	3	0	4	2
Visual disturbances	0	0	0	1	0	0	1	0	1	1
Shakiness or jumpiness	0	0	0	0	0	0	0	1	3	1
Total	13	18	12	16	15	16	20	13	60	63

*B, Benadryl.
†P, Pyribenzamine or tripelennamine.

Of the group of fifty-two subjects who received Benadryl, 77 per cent of the total exhibited some evidence of toxicity, and among the fifty-two persons given tripelennamine only, 67 per cent developed undesirable reactions. An analysis of the results obtained with the thirty-eight individuals who received corresponding doses of both drugs for identical periods of time (Table II, Fig. 1) confirmed the findings obtained when entirely different groups of subjects were employed for the testing (Table I). Six subjects, in whom each of the drugs was employed in daily doses of 150, 300, 450, and 600 mg., respectively, for a period of one week or more, did not develop any manifestations of toxicity which persisted or became chronic following the discontinuance of either or both drugs. It was definitely demonstrated that with small doses of each drug, signs

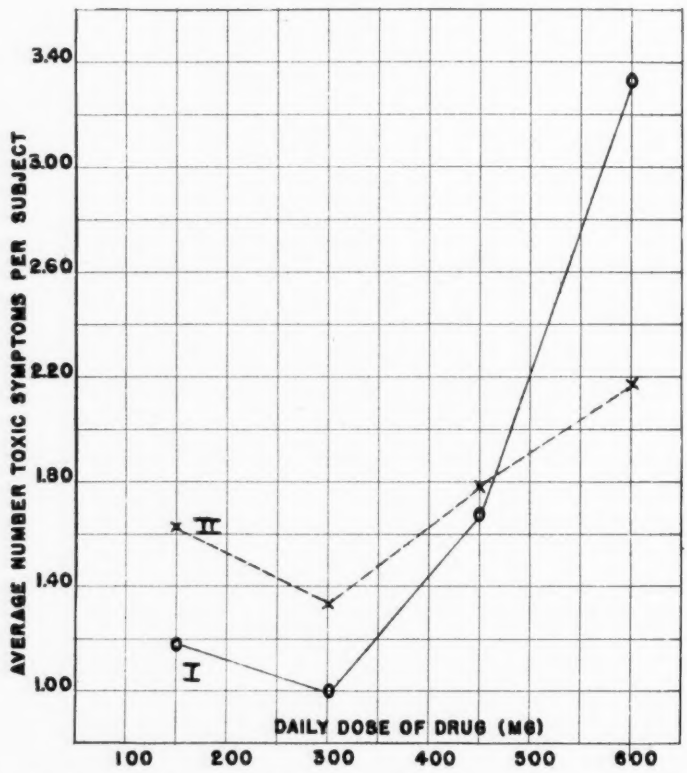

Fig. 1.—Toxic symptoms produced in a group of thirty-eight subjects following the oral ingestion of Benadryl (I) and Pyribenzamine (II), respectively, for periods of one week or longer at each of the dosage levels indicated.

and symptoms of toxicity were frequent, but mild in degree; both drugs were well tolerated. Drowsiness was more common in connection with the administration of Benadryl, and dryness of the mouth, with tripelennamine. With Benadryl, a tolerance against drowsiness usually developed in a few days, except with the use of the largest doses when cerebral manifestations became severe. When tripelennamine was used dryness of the mouth was common and increased in intensity as the daily dose was augmented. Regardless of the amount of drug

employed, within the limits noted, the majority of the other side reactions to tripelennamine, such as drowsiness, remained approximately the same. In other words, the tendency to establish tolerance was less than with Benadryl.

DISCUSSION

Both experimentally and clinically tripelennamine and Benadryl have been demonstrated to possess qualities powerfully antagonistic to histamine. Upon this common feature much of their efficacy in the treatment of allergic disease appears to rest. In addition to this, Benadryl shows a minor but still powerful hyoscine-like action not fully shared by Pyribenzamine. This provides, in addition to the antihistaminic effect, a mild sedative action which is often desirable in the control of many hypersensitive reactions.

Opportunity has been afforded to utilize human subjects for determining the comparative toxicity of Benadryl and tripelennamine. The facts that a representative number of these individuals received each of the two drugs at different times and that all observations were checked by the same techniques and personnel lend added emphasis to certain points brought out by an analysis of the data.

1. The high incidence of side effects. All side effects were recorded, regardless of their severity. When lower levels of dosage (150 and 300 mg.) were used, side effects rarely precluded the continued use of the drug. However, their frequency prompts a continued search for more nearly ideal agents in the management of the allergic reactions due to histamine.

2. The nature and percentage of side effects produced by each of the two drugs. It is clear from the present study that the intensity and number of cerebral manifestations is greater with Benadryl than with Pyribenzamine. It is equally evident that symptoms referable to the gastrointestinal tract are much more frequent with tripelennamine than with Benadryl. When the over-all incidence of all reactions is assessed, there is little difference between the two drugs at lower levels of dosage, that is 150 to 300 mg. daily. Indeed, the greater drowsiness caused by Benadryl is often of therapeutic importance, particularly in the itching dermatoses and in asthma. However, when higher levels of dosage (450 to 600 mg.) are employed, Benadryl is decidedly the more toxic of the two drugs (Fig. 1) and may cause confusional states not unlike those seen following alcoholic excesses.

From the present studies it seems that far too much emphasis has been placed upon the relatively high incidence of reactions to Benadryl, for instance 75 per cent,[3] as compared with the infrequency of reactions to Pyribenzamine, for instance 25 per cent or less.[3] Indeed, in the usual range of therapeutic doses (from 150 to 300 mg. daily) a critical study shows that this wide difference does not exist. Moreover, many of the unpleasant symptoms produced by Benadryl in such amounts are actually accounted for by its sedative action, which may sometimes be gainfully employed in therapy. At higher levels of dosage Benadryl becomes definitely the more toxic of the two antihistaminic substances under

discussion (Fig. 1) and should be used at such levels only when the patient can be under more or less constant surveillance. The gastrointestinal irritation of tripelennamine at similarly high levels of dosage usually precludes its use in the majority of patients.

3. Is there a direct relationship between toxic reactions and the antihistaminic effect? If this question is answered in the affirmative, one would expect that therapeutic effectiveness and toxicity would vary directly. However this is not the case. It seems likely, therefore, that the antihistaminic factor is not responsible for the untoward symptoms, but that some other property of each of these drugs plays a dominant role in producing side effects. This apparent dissociation between antihistaminic and toxic properties of these drugs furnishes a real incentive to continue the search for agents which will possess the antihistaminic factor to the complete or nearly complete exclusion of the toxic factor.

SUMMARY AND CONCLUSIONS

One hundred forty-two subjects without allergic disease or known disturbances of the autonomic nervous system were given Benadryl and tripelennamine (Pyribenzamine), respectively, in doses ranging from 150 to 600 mg. daily.

Of 52 subjects to whom tripelennamine alone was given, thirty-five, or 67 per cent, developed one or more toxic symptoms. Of an equal number of subjects to whom Benadryl alone was administered, forty, or 77 per cent, showed some type of untoward reaction.

In thirty-eight patients the effects of both Benadryl and tripelennamine were determined and comparisons of the effects of the two drugs were made on a weight for weight basis. The total number of reactions to Benadryl was sixty, and to tripelennamine, sixty-three. When 450 mg. or less of the drug were given, Benadryl showed definitely fewer reactions than did Pyribenzamine, but above that figure Benadryl produced a decidedly higher incidence of reactions.

Benadryl produced a preponderance of its disturbances in the sensorium; tripelennamine showed a predominance of gastrointestinal manifestations.

Usually the side effects did not preclude the therapeutic application of either drug in daily doses of 300 mg. or less. With Benadryl, initially unpleasant responses may disappear after the drug is continued for several days.

Both drugs are highly useful antihistaminic substances in the control—not cure—of a number of allergic diseases. In the usual range of dosage the incidence of side reactions is approximately equal and their intensity so mild as rarely to preclude their continued use. Indeed, the cerebral depression caused by both drugs, but more especially by Benadryl, may frequently add to the range of therapeutic applicability.

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EVALUATION OF A NEW SEDATIVE DRUG (3,3-DIETHYL-2,4-DIOXOPIPERIDINE)

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WITH THE TECHNICAL ASSISTANCE OF MARTHA FINE GREENBERG, B.A.

MOST physicians are confronted daily with the need of sedative medication for many of their patients. The value of the bromides and barbiturates is firmly established. However, these drugs occasionally produce side reactions such as rashes, headache, and lassitude. Moreover, continued use of the barbiturates may lead to tolerance or addiction. In the light of the foregoing, a new sedative which is distinguished by clinical effectiveness and good tolerability deserves consideration. Consequently, we set out to evaluate such a compound (3,3-diethyl-2,4-dioxopiperidine) submitted to us under the designation of NU-1510 and later under the name of Sedulon.*

The pharmacology of this substance and its oxidation product, 3,3-diethyl-2,4-dioxotetrahydropyridine, was thoroughly explored by Koppanyi, Herwick, Linegar, and Foster.[3] According to these investigators both compounds, in appropriate doses, produce motor paralysis, muscular relaxation, loss of righting reflexes, and deep sleep in experimental animals. The onset of action with the piperidine derivative is somewhat slower and its effect weaker and longer lasting than with the pyridine compound. From the figures presented by these workers it appears that, administered intravenously to rabbits, the narcotic index (LD_{50}/ND_{50})† of the piperidine derivative is 3.42, that of the pyridine compound, 4.07, and that of barbital, 2.96. Thus, the relative order of safety was found to be: pyridine derivative > piperidine compound > barbital. There were no significant changes in hemoglobin, erythrocyte count, leucocyte count, or differential count when daily doses of the pyridine derivative and the piperidine compound, respectively, were fed to rabbits for several weeks.

3,3-Diethyl-2,4-dioxotetrahydropyridine was evaluated clinically by Polatin and Horwitz[7] in psychiatric cases and by Freed[1] in mentally normal patients requiring sedative-hypnotic medication. These workers found the pyridine derivative to be a mild but very effective somnifacient. As a daytime sedative it generally proved adequate in patients with nervous tension.

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Received for publication, Oct. 30, 1947.

*We are indebted to Hoffmann-La Roche, Inc., Nutley, N. J., for supplies of NU-1510 (Sedulon) in the form of scored tablets of 0.25 gram.

†LD_{50} is defined as the lethal dose for 50 per cent of the animals and ND_{50} as the narcotic dose which prevents a righting response in 50 per cent of the animals after the tails are pinched.

Because of its weaker and more prolonged action as evidenced in animal experiments, 3,3-diethyl-2,4-dioxopiperidine held promise of being at least as satisfactory as its oxidation product for daytime sedation, and it was in this direction that a study was instituted.

The clinical work is presented in Part I, which gives results in patients requiring sedative medication. Part II gives the work done in man on urinary excretion following the administration of 3,3-diethyl-2,4-dioxotetrahydropyridine and of 3,3-diethyl-2,4-dioxopiperidine.

PART I

By Aaron E. Parsonnet, Arthur Bernstein, and Emanuel Klosk

The initial program called for an intensive study of a small group of subjects. Twelve patients with a diagnosis of essential hypertension were chosen. There were five men, ranging in age from 49 to 64 years, and seven women, whose ages ranged from 27 to 56 years. Some of these patients had cardiac symptoms, such as angina of effort, dyspnea, and palpitation; others complained merely of the frequent headaches and nervous manifestations so often endured by the hypertensive patient. All of them had been taking ¼ to ½ grain of phenobarbital three or four times a day for periods ranging from several months to a number of years and were thus suited for a comparative study.

Phenobarbital and all other medication were discontinued. Sedulon was substituted and the original plan of studying these subjects over a period of approximately two months was followed with but minor deviations. It was explained to each patient that he was going to receive a drug which might have an effect on the blood pressure, but no mention was made of sedative action. Deliberately their attention was diverted and all questions related to the sedation attained were injected casually in the course of subsequent interviews. It was thought that there was no need for alternating Sedulon and placebo medication since phenobarbital, known to the patients as exerting a sedative effect, was replaced by a drug which they expected to have a hypotensive but not a sedative action. Consequently, any results in the latter direction were probably not conditioned by a psychologic influence.

Treatment of eleven subjects was instituted with doses of 0.125 Gm., but only in one did this amount suffice to induce good sedation. The other ten subjects complained of nervous tension on a total daily dose of 0.375 Gm. and this was, therefore, doubled. However, the increase was not ordered before these patients had been on the lower dose for from three to four weeks and the change was effected always at an interview when there was no complaint about inadequate sedation.

One patient did not return for re-examination after she was placed on 0.25 Gm. three times daily. Of the nine subjects who were followed further, seven derived good sedation from a total daily dose of 0.75 Gm.; and in two only fair sedation was induced. A twelfth patient received initially 0.25 Gm. three times a day and this was attended by satisfactory sedation.

The action of Sedulon commenced thirty to sixty minutes after its intake and lasted from three to four hours following each dose.. The effect was found

to be of a selective sedative nature without marked soporific action. This quality is an advantage since doses of phenobarbital which were productive of a similar degree of sedation commonly rendered the patients sleepy at the same time.

The patient deriving good sedation from only 0.375 Gm. per day complained of pruritus, which was first thought to be due to the medication. Withdrawal of the drug, however, failed to result in subsidence of the pruritus which was finally relieved by psychotherapeutic measures. Reinstitution of Sedulon was not attended by recurrence of the pruritus. Similarly, there were no ill effects in any of the other patients.

Blood pressure readings for all subjects were done at frequent intervals. As can be seen from the average values in Table I, there was no significant reduction in either the systolic or the diastolic blood pressure.

TABLE I. AVERAGED BLOOD PRESSURE READINGS AND HEMATOLOGIC FINDINGS IN TWELVE HYPERTENSIVE PATIENTS TREATED FOR FROM FOUR TO EIGHT WEEKS WITH 3,3-DIETHYL-2,4-DIOXOPIPERIDINE (SEDULON)

	BEFORE TREATMENT	AFTER TREATMENT	RANGE OF WEEKLY AVERAGES	
			MINIMUM	MAXIMUM
Blood pressure	192/108	184/107	--	--
Hemoglobin (per cent) (Sahli)	91	96	91	98
Blood cell counts				
Erythrocytes (millions/c.mm.)	4.46	4.46	4.37	5.18
Leucocytes (millions/c.mm.)	7.64	8.13	7.04	8.73
Polymorphs (per cent)	59.4	61.8	54.0	66.2
Lymphocytes (per cent)	30.4	28.6	22.7	32.3
Monocytes (per cent)	7.4	7.4	6.5	9.0
Eosinophils (per cent)	2.7	2.6	2.6	3.5

Urinalyses carried out every week did not reveal any changes as compared with the premedication findings.

A special effort was made to determine the effect, if any, of the prolonged treatment with Sedulon on the blood picture. Hemoglobin determinations and red, white, and differential counts were done for all patients initially and at weekly intervals. As can be seen from Table I, which lists the average findings before and after treatment as well as the range of weekly averages, there were no significant changes in the hemograms during this period of close observation which included eight blood counts for most of the patients.

These gratifying results prompted us to extend the initial program.

Sedulon was thus continued in eight of the original group of twelve patients (Table II). Including the initial trial period, these eight subjects remained on the experimental drug for from six months to almost one year. In Patient 4 a total daily dose of 0.375 Gm. continued to induce good sedation for nearly eleven months, that is, up to the time the patient last reported for re-examination. The other seven subjects, each of whom received 0.25 Gm. three times a day, continued to derive the same degree of sedation during this period of extended trial as they did initially; that is to say, in Patients 1, 7, 9, 10, 11, and 12 good sedation was induced, and fair sedation resulted in Patient 8. Ill effects were absent and none of the subjects developed signs of tolerance or addiction.

TABLE II. BLOOD STUDIES IN EIGHT HYPERTENSIVE PATIENTS RECEIVING SEDULON FOR FROM SIX MONTHS TO ALMOST ONE YEAR

PATIENT	MEDICATION (MO.)	HEMOGLOBIN (PER CENT) (SAHLI)	ERYTHROCYTE COUNT (MILLIONS/C.MM.)	LEUCOCYTE COUNT (THOUSANDS/C.MM.)
1	0	92	4.45	6.45
	7⅓	96	4.20	5.80
	9¾	102	5.00	10.20
4	0	100	4.47	6.35
	8	84	3.67	6.95
	10¾	87	3.80	6.25
7	0	95	4.45	6.30
	6⅓	93	4.37	4.40
	10⅓	88	4.08	4.80
	11¾	90	4.34	5.20
8	0	86	4.03	7.25
	7	100	4.85	14.10
9	0	104	5.23	7.35
	6¾	86	4.30	8.40
	7¼	84	4.34	9.40
	9¾	85	4.40	5.50
	10½	94	4.41	7.45
10	0	90	4.39	6.20
	7¼	87	3.84	5.25
	10¼	93	4.35	5.00
11	0	87	4.54	7.50
	5¼	86	4.13	7.30
	7½	84	4.30	8.20
12	0	75	4.17	8.00
	6	81	4.30	6.20

0, The figures listed are premedication findings.

During the period of extended trial, hemograms were done for all eight patients at irregular intervals. Table II correlates some of the premedication blood findings and the findings after several months of medication. There occurred a decrease in the hemoglobin values and the erythrocyte counts in two patients (Patients 4 and 9), and a drop in hemoglobin values as well as in the red and white cell counts in one patient (Patient 7). However, Patient 4 was on a rice diet at the time of the shift in the red blood picture and Patient 7 underwent a cholecystectomy in May, 1947. In the former, reinstitution of a normal diet was followed by an increase in both the hemoglobin value and the red cell count; and all the values of the last count recorded for Patient 7, at a time when she had completely recovered from the operation, approximated the original findings. As for Patient 9, the erythrocyte count had dropped from 5,230,000 to 4,300,000 and the hemoglobin value had decreased from 104 to 86 per cent after more than six months of Sedulon intake. However, there was no further reduction in the number of red blood cells during the ensuing four months in spite of continued Sedulon medication, and the hemoglobin value increased appreciably during this time. It is concluded that none of these changes is attributable to the administration of the drug.

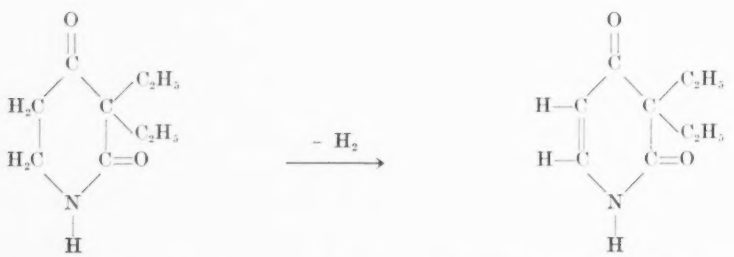
In further extension of the original program, the preparation was given to fifty patients complaining of nervousness, irritability, or emotional instability. In this group no blood studies were performed, but each patient was seen fairly regularly. The dosage was 0.25 Gm. three times daily and most of these fifty

patients have been taking the drug for from three to five months. While good or fair sedation resulted from this regimen, generally the patients were not over-sedated. However, in one patient it became necessary to reduce the dosage to 0.125 Gm. three times a day, and this dose was attended by satisfactory sedation. One patient developed some dizziness and nausea after taking the drug for about one month. Twenty-four hours after discontinuation of the medication, these symptoms subsided and did not reappear when Sedulon was reinstituted forty-eight hours later. However, since this patient was apprehensive about continuing the drug, it was stopped several days thereafter. Among the fifty subjects of this group there was one patient who had previously developed dermatitis from the use of phenobarbital. In contrast, Sedulon, which she has been taking for three months, is tolerated without untoward effects.

PART II

By Erich Hirschberg, Saul H. Rubin, and Leo A. Pirk

As was shown by Krautwald and co-workers,[5] there are no indications that 3,3-diethyl-2,4-dioxopiperidine is excreted unchanged in the urine when this compound is fed to dogs at a dosage level of 200 mg. per kilogram. However, these workers did recover between 7 and 8 per cent of the amounts of the administered piperidine derivative in the form of its oxidation product—3,3-diethyl-2,4-dioxotetrahydropyridine. The following structural formulas illustrate this transformation of the piperidine into the pyridine compound.



3,3-Diethyl-2,4-dioxopiperidine (Sedulon)

3,3-Diethyl-2,4-dioxotetrahydropyridine (NU-903)

With a new fluorometric method[2] (a modification of that described by Kubli and Schmidt[4]) for the determination of this pyridine derivative in urine at our command, it was decided to study its renal elimination in man following the administration of 3,3-diethyl-2,4-dioxopiperidine. It seemed of particular interest to compare the amounts of the pyridine derivative excreted after the intake of the piperidine compound on the one hand, with those following the administration of similar doses of the pyridine compound on the other.*

*For the sake of simplicity 3,3,-diethyl-2,4-dioxopiperidine is referred to as Sedulon, and 3,3-diethyl-2,4-dioxotetrahydropyridine as NU-903, the designation under which the latter compound was supplied by Hoffmann-La Roche, Inc., Nutley, N. J.

Four normal, male volunteers (physicians) ranging in age from 24 to 32 years were chosen. Collection of twenty-four-hour urine specimens was instituted seven days prior to the initiation of the excretion experiment proper and continued daily for five weeks. On the eighth evening each subject received 0.6 Gm. of NU-903 and after one week without further medication this dose was repeated. Again following a medication-free interval of seven days, 0.75 Gm. of Sedulon was given to each of the volunteers and a second identical dose was given at the end of the fourth week, with subsequent collection of urine specimens for another week.

Amber bottles, containing approximately 5 c.c. of toluene, were used for pooling the daily voidings of each subject. In carrying out the estimations of the urinary excretion of NU-903 in all the specimens thus obtained, the method described by Hirschberg and co-workers[2] was used. This technique is based on the measurement of the comparative fluorescence of urines, at suitable dilutions, in the absence and presence of hydroxylamine. The difference in intensity between total fluorescence (without hydroxylamine treatment) and residual fluorescence (with hydroxylamine treatment) yields a measure of the concentration of Nu-903 in urine. Sedulon is nonfluorescent under these conditions.

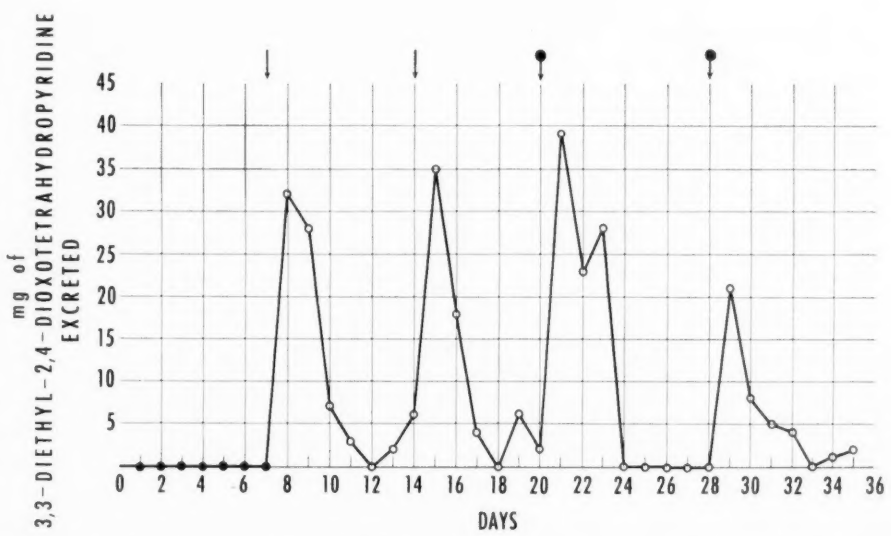


Fig. 1.—Urinary excretion of 3.3-diethyl-2,4-dioxotetrahydropyridine (NU-903) for Subject E. K. receiving, at weekly intervals, two single doses of 0.6 Gm. of 3,3-diethyl-2,4-dioxotetrahydropyridine (indicated by arrows) and two single doses of 0.75 Gm. of 3,3-diethyl-2,4-dioxopiperidine (Sedulon) (indicated by arrows with dots). The initial estimation was carried out seven days prior to the administration of the first dose of NU-903, and subsequent assays were performed daily for five weeks.

Urine specimens of persons who had not received either the pyridine or the piperidine compound were found to show some difference in intensity of total fluorescence and residual fluorescence after hydroxylamine treatment. This observation was the reason for testing seven premedication voidings of each of the four subjects. Thus, average correction factors for hydroxylamine-reactive

substances exhibiting irrelevant fluorescence were obtained. Calculated in milligrams of NU-903 per day, these correction factors were:

SUBJECT	
E. K.	3.9 ± S.D. 4.3
M. L.	6.8 ± S.D. 3.3
D. S.	−0.6 ± S.D. 6.0
E. S.	7.7 ± S.D. 3.9

It can be seen that the amounts of hydroxylamine-reactive substances excreted differed from subject to subject; in one subject (D. S.) the fluorescence of the hydroxylamine-treated specimens was even slightly greater than that of the untreated urine specimens. However, in the individual subjects the quantities of irrelevant interfering substances were sufficiently constant from day to day to permit the use of the correction factors. Consequently all findings were corrected accordingly.

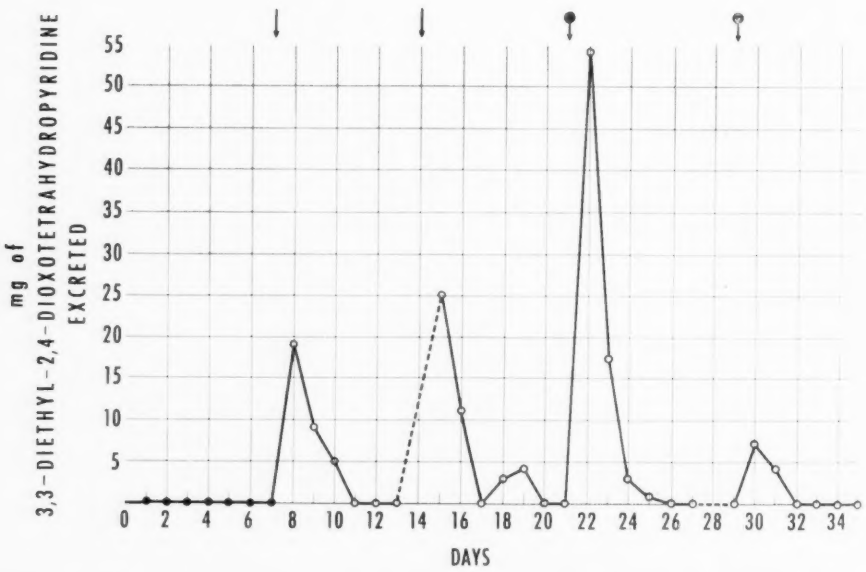


Fig. 2.—Urinary excretion of 3,3-diethyl-2,4-dioxotetrahydropyridine (NU-903) for Subject D. S. receiving, at weekly intervals, two single doses of 0.6 Gm. of 3,3-diethyl-2,4-dioxotetrahydropyridine (indicated by arrows) and two single doses of 0.75 Gm. of 3,3-diethyl-2,4-dioxopiperidine (Sedulon) (indicated by arrows with dots). The initial estimation was carried out seven days prior to the administration of the first dose of NU-903, and subsequent assays were performed daily for five weeks with the exception of two days (indicated by broken lines) when the specimens were lost.

Figs. 1 and 2 illustrate the procedure and the daily excretion values of NU-903 during the five weeks of experimentation for two subjects. The first two periods shown demonstrate the excretion of the pyridine compound following its administration; the last two periods show the elimination of the pyridine derivative following the administration of the piperidine compound. As can be seen, significant quantities of NU-903 were also eliminated following the ingestion of Sedulon.

The total amounts of NU-903 excreted in seven-day observation periods by four subjects following each of two doses of NU-903 and two doses of Sedulon are recorded in Table III.

It can be seen from Figs. 1 and 2 that both drugs are excreted rapidly, with the main portions of the eliminated amounts of NU-903 appearing in the first and second twenty-four hour urine specimens collected after the intake of both compounds. Three to four days following the individual doses, only negligible quantities of NU-903, if any, were detectable in the urine. Rapid elimination was observed also in the two other subjects.

TABLE III. URINARY EXCRETION OF NU-903 FOR FOUR NORMAL SUBJECTS AFTER SINGLE DOSES OF 0.6 GM. OF NU-903 AND AFTER SINGLE DOSES OF 0.75 GM. OF 3,3-DIETHYL-2,4-DIOXOPIPERIDINE (SEDULON)

SUBJECT	E_1	E_2	AVERAGE	E_3	E_4	AVERAGE
E. K.	77	65	71	90	41	66
M. L.	66	41	54	84	15	50
D. S.	33	43	38	75	11	43
E. S.	60	51	56	43	40	42
Average			55			50

E_1. The total excretion of NU-903 in milligrams following the first dose of NU-903.
E_2. The total excretion of NU-903 in milligrams following the second dose of NU-903.
E_3. The total excretion of NU-903 in milligrams following the first dose of Sedulon.
E_4. The total excretion of NU-903 in milligrams following the second dose of Sedulon.

As appears from Table III, the total excretion values after each of the eight individual doses of NU-903 varied considerably, ranging from 33 to 77 milligrams. However, in a given subject the total excretion values recorded following the two individual doses of the pyridine compound were in fair agreement. For instance, for Subject D. S., these values were 33 and 43 mg., respectively. The greatest difference was observed in subject M. L., who, following the second dose of NU-903, excreted 38 per cent less than after the first dose.

It can be seen likewise from Table III that the total excretion values of NU-903 following each of the eight individual doses of Sedulon ranged from 11 to 90 milligrams. Thus, these total excretion values varied even more than those recorded after the intake of NU-903. Moreover, the total amounts of the pyridine compound eliminated by a given subject following the two individual doses of Sedulon differed appreciably. For example, Subject D. S., whose excretion values after the two doses of NU-903 were in fair agreement, excreted 11 mg. of NU-903 following the second dose of Sedulon as compared with 75 mg. after the first dose, a reduction of 85 per cent.

In general the average of the two excretion values recorded for a given person following the administration of NU-903 was somewhat higher than the average of the two excretion values for that subject after the intake of Sedulon. The mean value of the average amounts of NU-903 excreted following 0.6 Gm. of NU-903 was 55 mg. or 9.2 per cent of the ingested dose, and the mean value of the average amounts of NU-903 eliminated after 0.75 Gm. of Sedulon was 50 mg. or 6.7 per cent. It seems that the elimination of NU-903 following the intake of this drug is slightly greater than after the ingestion of the piperidine compound.

The urinary excretion of NU-903 following administration of this compound was studied in man by Polatin and co-workers.[6] These investigators, too, reported that following a single dose of 0.6 Gm. the excretion level dropped promptly. For the six psychiatric patients whom they studied, the mean value of the total amounts eliminated over four days was 61 mg. or 10.1 per cent of the ingested amount. This is in good agreement with the present findings.

Similarly, the extent of elimination of the pyridine compound reported by Krautwald and associates[5] in dogs after the administration of 3,3-diethyl-2,4-dioxopiperidine approximates closely that determined for the four subjects in the present investigation; the excretion was 7 to 8 per cent of the amount fed to the animals as compared with a mean value of 6.7 per cent of the dose given to the human subjects.

Since the average excretion of NU-903 following the intake of this drug on the one hand and after the ingestion of Sedulon on the other appears to be of the same order of magnitude, it may be tentatively concluded that most or all of the piperidine compound administered is converted into the pyridine derivative.

The oxidation of the piperidine compound in the organism is suggestive of its mode of action. The belief that transformation of 3,3-diethyl-2,4-dioxopiperidine to the corresponding pyridine compound precedes its action is supported by both the somewhat slower onset and the longer duration of the effects of the former.[3]

SUMMARY OF PARTS I AND II

A new sedative drug, 3,3-diethyl-2,4-dioxopiperidine (Sedulon), was evaluated clinically in a total of sixty-two patients requiring sedation, twelve of whom had hypertension. Eleven of these subjects received 0.125 Gm. three times a day as the initial dose, but only one derived good sedation at this dosage level. With a dosage of 0.25 Gm. three times daily, good sedation was obtained in nearly all of the sixty-two cases. The effect of prolonged medication on the blood picture was studied in the twelve hypertensive patients receiving total daily doses of 0.375 and/or 0.75 Gm. for two months. Hemograms at weekly intervals showed no significant changes in either the red or the white blood picture. In eight of these patients, Sedulon was continued so that they received the drug for from six months to almost one year with no untoward effect on the blood picture. In none of the sixty-two cases was the administration of Sedulon attended by untoward effects. The drug failed to reduce the blood pressure in the twelve patients with hypertension.

Both Sedulon and its dehydrogenation product, 3,3-diethyl-2,4-dioxotetrahydropyridine (NU-903), are rapidly excreted in the urine as NU-903. Four human subjects excreted an average of 9.2 per cent of each of two doses of 0.6 Gm. of NU-903 taken seven days apart. Subsequently, they excreted average amounts of NU-903 equivalent to 6.7 per cent of each of two doses of 0.75 Gm. of Sedulon taken at weekly intervals.

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VARIATIONS IN DERMAL ABSORPTION WITH AGE

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THE activity of various bodily processes differs with age. It should be anticipated, therefore, that the rate or pattern of absorption of fluids from the skin will reflect these variations.*

It is the purpose of this report to describe definite variations in the absorption of a dye from an intradermal site of injection, the variation apparently being related chiefly to the age of the subject rather than to specific disease entities.

A review of the literature reveals that various aspects of this problem have received considerable attention. McClure and Aldrich[1, 2] utilized the disappearance time of intradermally injected normal saline solution as a test of edema in nephritic children. They observed a more rapid disappearance (to palpation) of the wheal than in normal controls. This test was extended to a variety of pathologic conditions. A shorter disappearance time was observed in scarlet fever and diphtheria,[3, 4] lobar pneumonia,[5] jaundice,[6] tuberculosis,[7] serum sickness,[8] toxemia of pregnancy,[9] and peripheral circulatory deficiency.[10-13] The disappearance time was found to be shorter in thyrotoxicosis[14] and prolonged in myxedema.[15] The work of Aldrich and McClure on children was confirmed by Feldman and Reifsneider[16] in adult nephritic patients. Berg[17] noted faster rate of disappearance in diabetic subjects, paralleling the severity of the diabetes, but this could not be confirmed by Leivy and Rynes.[18] Olmstead[19] in a study of children with heart disease, found the test of prognostic value in severely ill or toxic patients.

Thompson[15] stated that the circulation had little to do with dermal absorption of saline solution and concluded that the actual mechanism was unknown. Olmstead[19] also advanced this view, noting that disappearance time post mortem was not increased. White and Irvine-Jones[20] likewise concluded that circulation played no part, on the basis of their observations of the effect of altered blood flow, temperature changes, and adrenalin. They did not agree with Olmstead, however, in regard to an unaltered disappearance time post mortem.

White and Irvine-Jones[20] and Taylor[21] found intradermally injected fluids in the corium. McMaster and Hudack[22-24] who studied intradermally injected vital dyes in animals and humans, stated that intradermal injections are largely intralymphatic, the dyes entering lymphatics injured by the injecting needle. They observed that the dyes gradually spread through the superficial lymphatics,

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The Department is in part supported by the Michael Reese Research Foundation.
Aided by a grant from the A. B. Kuppenheimer Fund.
Received for publication, Jan. 14, 1948.

*Our attention was drawn to this problem by observations of Dr. H. Sorter from this hospital that blood penicillin levels (following intramuscular administration) frequently were lower initially and higher late in old patients than in young patients.

and stated that differences in the spread were due to differences in skin texture in different subjects which caused the needle either to fail to reach a lymphatic plexus or to penetrate beyond it. In either case, however, the dyes reach large, deeper lymphatics draining the injected area. Factors increasing lymph flow increased the rate of dye diffusion. Hudack and McMaster[24] pointed out that any local injection soon becomes a general one, since the injected material enters the blood stream rapidly. This is borne out by the appearance of phenol red in the urine within fifteen minutes after intradermal injection.[2] White and Irvine-Jones[20] were able to dismiss certain factors, such as hydrogen ion concentration, potassium/calcium balance, and osmotic pressure, as being of significance in the absorption of normal saline solution from the skin. They felt that the disappearance of the intradermal wheal was due simply to diffusion and, although they observed that artificially produced edema differed from nephritic or cardiac edema in that it did not produce an increased disappearance rate, they were unable to find the effective absorptive factor. In this connection it is interesting that McMaster[22] found lymph stasis in cardiac edema and increased lymph flow in nephritic edema, although in both instances the lymphatics are widened. In a recent article, Kurzrok and co-workers[25] referred to the work of Hoffman and Duran-Reynals on a factor which increased intradermal spread of various substances. Originally found in testicular extract, it was also found in certain bacteria, snake venom, and malignant tissue. This spreading factor, acting on connective tissue by hydrolysis of hyaluronic acid salts, is an enzyme, hyaluronidase. These authors concluded that the factor (enzyme or enzymes) causing diffusion in the dermis is identical with that exhibiting hyaluronidase activity.

SUBJECTS AND METHODS

The subjects were not selected except that, due to the nature of the test, most were of the white race. Sex distribution was approximately equal. No severely ill patient was used and most were well enough to be either ambulatory or semiambulatory. One hundred and one of the subjects could be considered normal in regard to cardiovascular system and skin. This group included a number of subjects who were either in normal health (hospital staff) or could be assumed to be healthy, such as surgical patients hospitalized for elective operative procedures. The conditions of the rest of the subjects (26 per cent) were such as would be encountered in the medical and surgical wards of any large general hospital. The experimental data obtained on these patients fell within the range of the normal controls in each group, and it was apparent upon analysis of all data that age rather than a particular disease was the chief determining factor in dermal absorption. On the basis of the accumulated data it was seen that the entire series could be divided into groups, each covering a period of fifteen years. The data on each age group were compared for statistically significant differences by the chi square test (Fisher), using a 5 per cent level of significance.* Under Results we refer to this analysis when differences are called statistically significant.

One-tenth cubic centimeter of a sterile solution of Evans blue in normal saline (0.05 mg. per cubic centimeter) was injected intracutaneously on the middle third of the volar surface of the forearm, using a short No. 25 hypodermic needle and a tuberculin syringe. One of us (J. S.) made all injections and readings. Four periodic measurements were made on each subject. This was done by placing a strip of clear celluloid film over the wheal or dye stain and marking out the pattern. It was usually necessary to emphasize the dye outlines with

*We are obliged to Dr. H. Silverstone for help in the statistical work.

a pen before applying the film. Lighting conditions and room temperatures were standardized as much as possible. The area of the tracing on the film was measured with a planimeter. Readings were made immediately after injection, after an interval of from one and five-tenths to six hours, usually at four hours, and at twenty-four and forty-eight hours. Because the average time after injection of the second reading was four hours, it will be referred to as such.

Evans blue is a complex organic compound ($C_{34}H_{26}O_{14}N_6S_4Na_4$) with a molecular weight of 962.82 commonly employed in determination of blood volume, where the dye combines with serum protein, especially the albumin fraction. It is a blue powder, soluble in water but not in alcohol or ether. The proper dye strength was arrived at after trial and error experimentation to determine which concentration would produce a satisfactory color of the skin and would disappear within a reasonable time.

Observations indicate that strictly intracutaneous injection is next to impossible to achieve,[20, 21] but because of the widespread use and understanding of the term we have continued to employ it. The presence of a blanched, raised wheal with a surface appearance of orange peel was taken to indicate a satisfactory injection. Within a period of from a few minutes to four or more hours the wheal disappeared, leaving a blue circular or oval stain with well-circumscribed edges. The stain increased or decreased in size at successive observations, eventually fading to a point where accurate measurement was impossible. This end point exhibited a variability ranging from a few hours in one instance to forty-eight hours or more in many others. In most of the adults the dye was either totally absent or too faint to be measured at forty-eight hours. The reading was consequently recorded either as trace or absent. In the discussion and tables the term wheal is employed for simplicity as synonymous with stain.

In view of the reported inconstancy of the rate of dermal absorption in the same individual[20] we made no attempt to duplicate all results, except in a few instances. Where repeat tests were done, however, we noted that the pattern of absorption was always identical.

RESULTS

The results showed a number of differences in the absorption of intradermally injected Evans blue in different age groups. The initial size of the wheal is analyzed in Table I, which indicates a distinct tendency for the average size of the initial wheal to increase with increasing age. The data in Table II demonstrate a steadily increasing number of cases in the groups above 30 years of age having an initial wheal area greater than 0.75 square centimeter. The latter figure represents the average of the initial readings in all groups. While there is little significant change between any group and its immediate neighbors, the differences between more widely separated groups have statistical significance.

TABLE I. DISTRIBUTION OF WHEAL AREAS AT TIME OF INJECTION

AGE GROUP (YR.)	AVERAGE AGE OF GROUP (YR.)	AREA OF WHEAL IN SQUARE CENTIMETERS										NUMBER OF CASES IN EACH AGE GROUP
		0.4	0.5	0.6	0.7	0.8	0.9	1.0	1.1	1.2	1.3	1.4
		NUMBER OF CASES										
0-15	6.5	5	3	3	1	1						13
16-30	22.3	2	1	6	12	4	4	1				30
31-45	38.1		3	2	4	5	1	3				18
46-60	54.8			4	10	9	3	3	2	1		32
61-75	68.7	1	1	7	7	9	8	2	2	1		38
76-90	79.8		1		1	2	1	1				6
											Total	137

TABLE II. INITIAL WHEAL AREA

AGE GROUP (YR.)	PER CENT OF CASES WITH WHEAL AREA GREATER THAN 0.75 SQ. CM.
0-15	8
16-30	30
31-45	50
46-60	56
61-75	58
76-90	67

It is seen in Tables III and IV that the same general pattern continues at the end of four hours except in the 16- to 30-year age group where there is a trend toward a decrease in the wheal size, which, however, was found to have borderline statistical significance only.

TABLE III. DISTRIBUTION OF WHEAL AREAS FOUR HOURS AFTER INJECTION

AGE GROUP (YR.)	AREA OF WHEAL IN SQUARE CENTIMETERS																NUMBER OF CASES IN EACH AGE GROUP
	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0	1.1	1.2	1.3	1.4	1.5	1.6	1.7	1.8	1.9	
	NUMBER OF CASES																	
0-15			4	3	1	1	2	2										13
16-30	1	4	9	2	6	2	1			1		1	1					28
31-45		3		4	2	1	2			1		2	1				1	17
46-60		2	5	5	4	1	6	1	2		1	2	3					32
61-75		1	2	8	3	6	4	6	1	2	1	1	2			1		38
76-90				2		1	2				1							6

TABLE IV. WHEAL AREA FOUR HOURS AFTER INJECTION

AGE GROUP (YR.)	PER CENT OF CASES WITH WHEAL AREA GREATER THAN 0.75 SQ. CM.
0-15	39
16-30	20
31-45	44
46-60	50
61-75	63
76-90	67

It is further apparent, as shown in Table V, that visible dye remains in the injected area of a significantly larger number of persons in the younger age groups. The two groups from 10 to 14 and from 15 to 29 years of age had visible dye left in 90 to 97 per cent of the cases, while the two older groups had visible dye in 57 to 79 per cent.

TABLE V. NUMBER AND PER CENT OF CASES WITH DYE SEEN AT TWENTY-FOUR AND FORTY-EIGHT HOUR READINGS

	AGE GROUP (YR.)			
	10-14	15-29	30-39	60 AND OVER
Number of cases in group	14	29	48	47
24-Hour reading (%)	93	97	71	79
48-Hour reading (%)	93	90	57	58

DISCUSSION

In young persons the initial wheal was smaller than in older persons (Tables I to IV). On the other hand, the dye remained longer in the skin of the younger subjects (Table V). Usually the size of the initial wheal increased in young persons before it disappeared, while in older subjects it usually decreased steadily. These results are not readily explained. Lymphatic absorption may play a larger role than absorption into the venous blood.[24] It has been stated that the number of skin capillaries diminishes with age,[27] and one might assume that the number of lymphatic vessels diminishes likewise. Thus, in younger subjects lymphatic absorption may be greater in the initial phase of skin absorption than in older subjects.

Differences in texture of the skin between old and young persons are so distinct that they may well explain some of the phenomena observed. In the young, skin turgor is high and there are more elastic fibers present. In the old, turgor is low, elastic tissue is reduced, the epidermis and the deeper layers of the skin atrophy, and the skin has folds and consequently a greater surface area. In our tests it was easy to inject 0.1 c.c. of dye solution into the skin of older persons, but in the youngest group it was hard to push the needle through the skin and much more pressure had to be exerted to push the fluid into the skin. On a physical basis these differences may explain the larger initial wheals in the older subjects and the smaller ones in the younger subjects. Also, a possibly more rapid initial absorption in the younger group may be explained by the higher initial pressure in the wheal. On the other hand, the longer secondary persistence of dye in the skin of the younger group (Table V) may be explained by the relatively smaller area of the wheal and a consequently smaller absorbing area in which the dye is contained. In the older subjects the ready spread of the dye opens a larger absorbing area; initial absorption may be poor due to fewer lymphatic vessels, lesser pressure, possibly less tissue damage, less motility of the skin, and consequently less lymphatic suction, but the secondary stage of absorption (twenty-four and forty-eight hours) may be faster, due to the greater area occupied by the dye.

We have discussed mechanical factors mainly. Chemical factors, such as differences in hyaluronic acid salts and hyaluronidase, in histamine liberation, in water and salt content of the skin, in proteins that bind the dye, in macrophages, and so on, of old as compared with younger people are even more unknown than physical factors.

SUMMARY

Differences in the absorption pattern of intradermally injected Evans blue in different age groups were demonstrated.

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FURTHER STUDIES ON THE WELTMANN SERUM COAGULATION REACTION

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IN 1942 one of us (M. K.)[1] presented a study on the clinical significance of the Weltmann serum coagulation reaction, using as subject material the results of the reaction as tested in over 1,100 patients. Baker[2] shortly thereafter reported further results from Kraemer's laboratory. The present report is a continuation of those studies, covering the Weltmann reaction in over 3,000 cases.

Weltmann,[3, 4] in 1930, described a serum coagulation reaction which has shown itself to be of considerable clinical significance. He found that when normal human blood serum was diluted with distilled water 1:50 and boiled, the proteins failed to coagulate. However, when a small amount of electrolyte such as calcium chloride, magnesium chloride, or barium chloride was added, coagulation occurred. In certain pathologic conditions Weltmann noted that the serum required greater than normal concentrations of electrolyte in order for protein coagulation to occur, while in other conditions less than the normal concentration of electrolyte was required for coagulation. If 0.1 ml. of normal serum was added to 5 ml. of .04 per cent calcium chloride solution and boiled, the protein coagulated. However, if the serum was added to .02 per cent calcium chloride, coagulation did not take place.

Exudative and inflammatory processes so altered the blood serum that coagulation of the protein occurred only in concentrations of calcium chloride higher than .04 per cent. Conversely, fibrosing processes so altered the serum that coagulation occurred in concentrations of calcium chloride lower than .04 per cent. From these findings Weltmann devised the relatively simple test described below.

Various theories were propounded to explain the mechanism of the Weltmann reaction. Alterations in the following factors, either singly or in combination, were suggested as the basis of the reaction: the pH of the calcium chloride solutions employed, the pH of the serum, the serum calcium concentration, the total blood globulin, the albumin-globulin ratio, and the blood fibrinogen.[5] Dees studied the reaction extensively[6-8] and suggested that the serum lipids are an important factor in the coagulation reaction. However, Scherlis and Levy[5] showed that the coagulation reaction is closely related to changes in the percentage of alpha globulin in the blood.

From the Presbyterian Hospital.
Read at the Meeting of the Eastern Section of the American Federation for Clinical Research, Cornell University, New York, Dec. 12, 1947.
All technical procedures were performed by Miss Margaret Palmer.
Received for publication, Dec. 29, 1947.

TECHNIQUE

Ten test tubes are set up in a rack and are numbered 1 to 10 from left to right. From a stock solution of .1 per cent calcium chloride ($CaCl_2.6H_2O$) measured amounts are pipetted into each tube starting with 5 ml. in the first tube and decreasing by 0.5 ml. in each tube so that tube 10 contains .5 milliliter. Sufficient distilled water is added so that each tube will contain a total of 5 ml. of solution, that is 4.5 ml. of water are added to tube 10, 4 ml. to tube 9, and so on in decreasing amounts to .5 ml. in tube 2. This procedure results in ten dilutions of calcium chloride ranging from .1 per cent in tube 1 down to .01 per cent in tube 10.

To each tube is then added 0.1 ml. of the blood serum to be tested. The serum must be unhemolyzed. After shaking, the tubes in their rack are placed in a boiling water bath for exactly fifteen minutes. The rack of tubes is then removed from the bath and the number of tubes in which coagulation has occurred is recorded. The coagulum appears as a flocculation. Coagulation normally appears in the tubes of higher concentration (Tubes 1 to 6) and not in the tubes of lower concentration (Tubes 7 to 10). The number of the tube containing the most dilute solution of calcium chloride in which coagulation has occurred gives the reading for the test. Thus, if coagulation occurs in tubes 1 through 6, the coagulation band is 6 (C. B. 6 or Weltmann 6). Care must be taken to determine the last tube in which *actual* coagulation has occurred, since clouding or turbidity may occur in two or three tubes more dilute than the significant tube. With normal human serum, coagulation usually occurs in the first 5 to 7 tubes (C. B. 5 or 6 or 7). When the coagulation band is less than 5, it is said to shift to the left, and when it is more than 7, it is said to shift to the right.

In actual practice we have simplified this technique to ease the burden on the technician without sacrificing accuracy. If coagulation occurs in any tube, by definition, it will also occur in every tube to the left of it (that is, in all higher concentrations). Therefore, tubes 1, 2, 9, and 10 are not routinely set up, since coagulation usually occurs in at least three tubes and rarely occurs in more than eight tubes. If, after the initial test is performed, no coagulation has occurred in tube 3, tubes 1 and 2 are set up separately and the serum sample is tested in these tubes. Conversely, if in the initial test coagulation has occurred in tube 8, tubes 9 and 10 are set up separately and the serum is tested in these tubes. For a single test this modification may seem of little value. However, when an average of five samples of serum are tested simultaneously in parallel rows of tubes as is done in our laboratory, the time and effort saved with this modification are considerable.

Interpretation of the Test.—Weltmann[3, 4] demonstrated that in the presence of an exudative or inflammatory lesion there was a shift to the left of the coagulation band and that in the presence of fibrotic lesions there was a shift to the right. Subsequent investigators have corroborated these findings.[6-11] However, the bulk of significant results appears to be confined to the cases in which a shift to the left was obtained. This is in agreement with our findings.

Previous writers have found the coagulation band of normal sera to be rather fixed at 6 or 7.[3, 7, 9] In our hands, however, we found we had to consider the normal range as varying from 5 to 7.[1] Wachstein[11] also designated a coagulation band of less than 5 as being significantly shortened, regarding a coagulation band of 5 as being "suggestively shortened."

CLINICAL STUDY

In Kraemer's[7] report, his results with the Weltmann reaction as performed on about 1,100 patients were summarized. The present report includes that material plus the results obtained with sera from about 1,870 additional patients, a total of 3,954 Weltmann tests having been performed on about 3,000 consecutive private patients.

During the period covered in this report, a Weltmann coagulation test as well as a complete blood count, urinalysis, and sedimentation rate (Westergren or Cutler) was performed at least once on each patient in addition to other indicated diagnostic procedures. Most of the patients presented gastrointestinal problems and it is in this group that we have been able to follow the results of the Weltmann reaction with the greatest degree of accuracy.

Of the 3,954 Weltmann tests performed, a total of 281 showed a coagulation band of 4 or less. It was possible to closely correlate the clinical or pathologic findings with the low Weltmann reactions in 237 of these cases. In the remaining 44 cases no such correlation was possible. This latter group will be discussed later.

In Table I are listed the disease entities which were associated with a significant number of shortened coagulation bands.

TABLE I

DIAGNOSIS	TOTAL NUMBER OF CASES	PERCENTAGE OF LOW WELTMANN REACTIONS
Regional ileitis	9	55.6
Diverticulitis	16	50.0
Gastric carcinoma	29	41.4
Ulcerative colitis	54	35.2
Gastric ulcer	50	32.0
Choledocholithiasis	10	30.0
Carcinoma of the colon	34	26.5
Carcinoma of the rectum	18	16.7
Cholecystitis, cholelithiasis	296	10.8
Duodenal ulcer	374	10.4

It will be noted that in 55.6 per cent of cases of regional ileitis the Weltmann was 4 or less, this being the highest percentage in our series. In 50 per cent of cases of diverticulitis coli and in 41.4 per cent of cases of proved gastric carcinoma, the Weltmann was 4 or less. Cases of ulcerative colitis, gastric ulcer, and choledocholithiasis also showed significant percentages of low Weltmanns, 35.2, 32, and 30 per cent, respectively. Conversely, cases of duodenal ulcer, cholecystitis, and cholelithiasis showed the lowest percentages of low Weltmanns, although it has been our experience that in cases of penetrating duodenal ulcer and in ulcer with obstruction the incidence of low Weltmann reactions has been

relatively high. These results appear to confirm the finding that the coagulation band is shortened in the presence of exudative or inflammatory lesions. Ulcerative colitis is characterized by marked exudative and ulcerative inflammation. Gastric ulcer, penetrating duodenal ulcer, and obstructive duodenal ulcers are generally associated with considerable inflammatory reactions, and choledocholithiasis is in many instances the cause of chronic low-grade inflammation in the biliary tree. With regard to ulcerative colitis one might take the view that an incidence of 35.2 per cent positive Weltmanns is relatively low, considering the characteristic pathology of the disease. It should be mentioned, therefore, that a number of the cases studied were quiescent at the time of study and have remained so.

Gastrointestinal malignancy, especially gastric malignancy, is often associated with secondary ulceration and inflammation, and the latter is probably responsible for the low Weltmann rather than the malignancy per se. We have observed a number of cases which would seem to substantiate this fact. Two of the cases will be cited briefly.

CASE 5919.—The patient complained of increasing constipation of one year's duration. Proctoscopy (June 6, 1946) revealed a small fungating growth six inches above the anus. The Weltmann (June 10, 1946) was 4. A barium enema (June 10, 1946) revealed a filling defect of the rectosigmoid; a chest plate made the same day showed metastatic infiltration of the right lower lobe. The primary growth was removed by abdomino-perineal resection. Three months later, although the pulmonary metastases had increased in size and number, the Weltmann (Sept. 19, 1946) was 6. These metastases were discrete and were associated with no inflammation or exudation; a low Weltmann was not expected once the ulcerating primary lesion was removed. On Dec. 24, 1947 the patient complained of pain in the left side of the chest on inspiration. A chest plate taken that day revealed a small pleural effusion. The Weltmann performed the same day was 3.

CASE 5660.—Four months after partial gastric resection for gastric carcinoma and six months after radical mastectomy for primary carcinoma of the breast, the Weltmann was 5 (July 27, 1946). Four months later (Nov. 16, 1946), the Weltmann was still 5. The patient was vomiting intermittently. In January, 1947, the patient developed signs of pleural effusion which proved to be metastatic in origin. The Weltmann (Jan. 16, 1947) was 1, an expected finding because of the appearance of the exudative lesion.

DISCUSSION

The Weltmann serum coagulation reaction appears to be of sufficient value as a laboratory diagnostic aid to warrant its routine use in gastroenterological diagnosis. Admittedly it is a nonspecific reaction, but this fact does not detract from its utility. In the presence of exudative or chronic inflammatory reaction the Weltmann coagulation band is shifted to the left in a significant number of instances. It has been our experience and that of others[5] that, in general, whenever there is a low Weltmann the sedimentation rate is elevated, but there is no correlation between these findings. With a Weltmann of 4 one may find a sedimentation rate of 20 or 85, whereas the same sedimentation rate may be noted with a Weltmann of 6 or 2. The sedimentation rate is often influenced by a number of factors, many of them of relatively minor nature, and it thereby loses diagnostic significance. The Weltmann reaction, however, when positive,

that is when 4 or less, usually indicates, in gastrointestinal complaints, an inflammatory lesion of serious import and rapidly reverts to normal when this lesion is healed or removed. It is on this account helpful in both diagnosis and prognosis. The following cases will illustrate this point.

CASE 6898.—On Oct. 6, 1947, the Weltmann was 4 and the sedimentation rate, 36. Posterior wall gastric ulcer and duodenal ulcer were found on x-ray examination. Gastroscopy revealed a benign lesion. On Nov. 18, 1947, after six weeks of treatment, the patient was asymptomatic with a Weltmann of 6 and a sedimentation rate of 46. The niche was still present but much smaller.

CASE 6542.—April 1, 1947, there was a history of recurrent epigastric pain of four years' duration which was relieved by food. The Weltmann was 4 and the sedimentation rate, 22. Gastrointestinal x-ray films showed no evidence of lesion but the patient was placed on an ulcer type of therapy. On May 20, 1947, there were no complaints; there was a weight gain of five pounds; the Weltmann was 7, the sedimentation rate 11. On June 17, 1947, there was occasional slight epigastric discomfort; the Weltmann was 4 and the sedimentation rate, 18. On Sept. 18, 1947, a posterior wall gastric ulcer was found on repeat x-ray study (confirmed at operation).

In 44 cases with positive Weltmanns we were unable to determine the cause of the positive reaction. Almost without exception this group consisted of patients who were seen only once and therefore an adequate diagnostic work-up and follow-up were not possible. More thorough study probably would have revealed the pathologic basis for the positive test in all or most of these patients, but the possibility of false positive reactions in this group cannot be ruled out. However, in the 237 patients in whom adequate study was performed, a small number of positive tests was obtained in inflammatory states such as acute severe gingivitis, acute tonsillitis, or acute sinusitis; with subsidence of the inflammation the Weltmann reactions in these instances rapidly reverted to normal. The incidence of actual false positive reactions, that is positive reaction in the absence of any demonstrable inflammatory or exudative lesion, must be very slight.

It would be unwise to attribute to the Weltmann reaction values which it does not have. Many cases of serious disease of the gastrointestinal tract have been diagnosed in the presence of a normal Weltmann. However, when the Weltmann has been positive, complete diagnostic work-up has usually revealed a lesion of importance. This has led us to the clinical assumption that a patient with a low Weltmann reaction has a serious illness until absolutely proved otherwise. In a number of instances the cause of a low Weltmann has been traced to important lesions outside the gastrointestinal tract after gastrointestinal work-up had revealed a lesion sufficient to explain the presenting symptoms but not sufficient to explain the low Weltmann reactions.

SUMMARY AND CONCLUSION

The Weltmann serum coagulation test and a modification for its simplification in routine use are described.

The results of the Weltmann test as performed in over 3,000 consecutive patients and the significance of the reactions have been analyzed.

The test appears to be of considerable value in indicating the presence of exudative or inflammatory disease of the gastrointestinal tract, including neoplastic lesions when the latter are accompanied by ulceration or exudation.

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ELECTROPHORETIC STUDY OF THE BLOOD SERUM FROM LYMPHOGRANULOMATOUS PATIENTS

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THE present investigation was undertaken in order to determine whether electrophoretic analysis of blood sera withdrawn from patients with Hodgkin's disease would demonstrate patterns peculiar to this disease. Such findings might be useful in diagnosis and prognosis and might throw light upon the nature of the illness. Impetus was given to our studies by recent electrophoretic investigations of human sera obtained from normal and diseased subjects indicating that the protein fraction reflected the state of the organism as a whole and that in one instance (in nephrosis) a strikingly characteristic curve was obtained.

EXPERIMENTAL

Materials and Methods.—Blood samples were withdrawn under sterile conditions from patients under observation and treatment at the Hodgkin's Disease Research Clinic of St. Vincent's Hospital. These samples were allowed to clot and were stored in the refrigerator until examination.* The diagnosis of Hodgkin's disease was in every instance proved by biopsy and in some instances further confirmed by necropsy.

For electrophoretic examination 5 to 10 ml. of blood serum were diluted with two volumes of sodium barbiturate-barbituric acid buffer of an ionic strength of 0.1 and a pH of about 8.5. The diluted serum was then dialyzed in cellophane tubing against 2 liters of the same buffer for two to three days in the refrigerator. Before placing the dialyzed serum in the electrophoretic apparatus, it was centrifuged in an angle centrifuge to sediment any suspended particles; the tall section analytic cell of Tiselius-Longsworth[3] of 11 ml. protein solution capacity was employed throughout. The apparatus was a commercial instrument closely based upon a design by Longsworth.[4] The electrophoretic diagrams were recorded photographically with the schlieren-scanning method.[4] Visual observations in the course of the experiments were made with the Svensson-Philpot cylinder lens-angular diaphragm technique.[3] The temperature of the thermostat was 1.3° C., the voltage gradient about 6.6 v. per centimeter, and the time of electrophoresis approximately 14,000 seconds.

As light source, a mercury high-pressure burner (G. E. H-4) was used in the case of clear and little-colored serum specimens, and a single filament tungsten source (G. E. 3.5 A./ 14 T., 10 V.) for the examination of colored or opalescent sera.

The hydrogen ion concentration and the conductivity of the solutions were measured with a glass electrode pH meter and an electronic conductivity bridge, respectively.

This investigation was aided by a grant from the Donner Foundation to Dr. Robert Chambers and Mr. Costa Grand, of the Department of Research Biology, New York University Washington Square College.

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Received for publication, Oct. 17, 1947.

*The electrophoretic experiments were performed in the Institute of Polymer Research, Department of Chemistry, Polytechnic Institute of Brooklyn.

For quantitative evaluation the original photographic negatives were enlarged about three times by projection and the enlarged patterns were traced by hand. The tracings were then measured with a polar planimeter after the areas had been divided by a vertical line through the minima and subdivisions assigned to the individual components.[3] Relative concentrations in per cent were computed in terms of the ratio of these areas to that of the total area under the curve after the stationary, anomalous boundaries had been eliminated from the diagram. In the majority of cases the calculations are based on the pattern recorded from the descending boundaries, but in two instances the ascending boundary patterns were also evaluated (see Tables I, II, and III). It is realized that the procedure here adopted disregards the small differences in the refractive increment of the various proteins of blood serum and also the contribution of protein to the boundary anomalies. It is felt, however, that in comparison with the other possible sources of error in electrophoretic determinations these factors are of only secondary importance.

The electrophoretic mobilities of the individual components appeared to be of the same order as those of normal serum, hence no systematic measurements of mobilities were undertaken.

In order to avoid any bias on the part of the investigators, the serum samples were submitted for examination without any clinical information. In some instances several specimens from the same patient, obtained after varying intervals of time, were studied in the electrophorectic apparatus.

The sera specimens examined were obtained from twenty-seven patients suffering from Hodgkin's disease, eighteen men and nine women. Duration of affliction and severity of manifestations were not alike in any two cases. The age range was from 13 to 61 years and the duration range from 1 to 5 years. There were further variations, namely in the rapidity of development of disease, in the state of nutrition of the patients, and so forth.

Observations and Results.—A total of thirty-three serum samples was examined electrophoretically under strictly comparable experimental conditions. The electrophoretic patterns obtained fell into three groups.

The first group (A) yielded diagrams somewhat similar to those of normal individuals. The albumin-globulin ratio was greater than unity and the absolute as well as the relative concentrations of the individual protein components, excepting the α-globulin fraction, were found to be near the limits of variation found previously for normal human serum both in this laboratory and by other investigators. The electrophoretic data pertaining to this group are compiled in Table I. For the purposes of comparison the mean values as determined by Dole and Braun[5] on the blood plasma of fifteen normal, young, adult male subjects under similar experimental conditions are included in Table I. The fibrinogen values are omitted because serum was used in the present study. This component amounts to approximately 5 per cent of the total area of the plasma protein pattern.

It will be noted that the values for the different serum components vary appreciably from case to case, as does also the albumin-globulin ratio. On the

TABLE I. ELECTROPHORETIC COMPOSITION OF SERA FROM LYMPHOGRANULOMATOUS PATIENTS IN GROUP A; THE VALUES HERE RECORDED ARE SIMILAR TO THOSE FOR NORMAL AND NEARLY NORMAL BLOOD SERUM

EXPERIMENT	AGE (YR.)	SEX	DURATION OF ILLNESS, ONSET TO EXPERIMENT	CLINICAL STATUS	A/G RATIO	RELATIVE CONCENTRATION IN PER CENT OF TOTAL AREA				
							GLOBULINS*			
						ALBUMIN	ALPHA-1	ALPHA-2	BETA	GAMMA
421	32	M	11 mo.	Good	1.1	56.7	4.73	12.5	11.3	14.8
422	21	M	5 yr.	Fair	1.7	62.4	6.14	14.8†	11.0	5.65
427	24	M	10 mo.	Good	1.6	61.3	5.24	7.75	15.1	10.5
429-a	33	M	24 mo.	Good	1.7	63.0	5.84	14.5	10.4	6.37
443-a	33	M	11 mo.	Good	1.1	51.9	5.7	11.3	15.6	15.4
457	29	M	2 yr.	Fair	1.0	50.0	7.0	15.3	16.4	11.3
467-a	13	M	6 mo.	Good	1.5	60.4	7.5	9.5	11.6	11.2
471	35	M	17 mo.	Good	1.2	55.1	4.6	10.2	12.5	17.8
467-b	13	M	7 mo.	Good	1.8	64.0	5.0	14.1	7.5	9.3
480-a	19	M	9 mo.	Good	1.4	58.3	6.9	13.7	7.9	13.3
483	29	M	25 mo.	Good	1.2	50.6	7.5	14.3	16.9	10.7
443-b	33	M	14 mo.	Good	1.4	62.0	5.7	11.3	5.6	15.4
474	27	M	12 mo.	Good	1.3	57.2	7.5	10.9	13.4	11.1
Mean value					1.4	57.9	6.1	12.3	11.9	11.8
Average deviation					±.2	±4.0	±.9	±2.0	±2.8	±2.8
Normal values for plasma (Dole and Braun[5])‡					1.53	60.3	4.6	7.2	12.1	11.0

*Relative concentration in per cent of total area, as calculated from diagrams obtained in the descending limb of the cell.

†Component split in two peaks.

‡These values represent the average for the ascending and descending limb of the cell. For serum these figures should be increased by about 5 per cent.

whole, the albumin-globulin ratios of the sera grouped together in Table I are somewhat lower and the relative concentrations of the α-2 and of the γ-globulin fractions are higher than the values reported by Dole and Braun for normal serum. This is true even if allowance is made for the absence of fibrinogen in the samples studied by us. How significant these deviations are, namely how far they may be due to the fact that these sera were all obtained from patients with Hodgkin's disease, cannot be stated at this time. The same holds true for the differences in quantitative composition of the specimen examined as experiment 467-a and that withdrawn from the same patient four weeks later and examined as Experiment 467-b. A pair of electrophoretic diagrams representative of this group of sera is reproduced in Fig. 1.

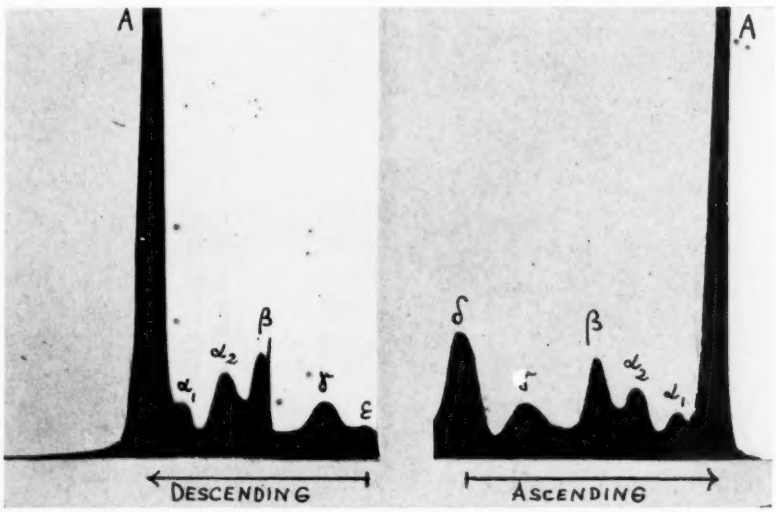

Fig. 1.—Electrophoretic diagram of serum from a patient with Hodgkin's disease, falling into the A group (Experiment 471, Table I): normal or nearly normal pattern as to general outline, absolute and relative concentration of components, and electrophoretic mobility.

The second group (B) of sera, listed in Table II, shows the following features in common. The α-globulin fraction is present in an amount which is significantly higher than in normal blood serum. The increase is limited either to the α-1 or the α-2 globulin component or it involves both of these protein fractions of high electrophoretic mobility.

With a single exception the albumin-globulin ratio is below unity. In some instances the β-globulin component is also increased in relative concentration (Experiments 381, 382, 419).

The significance of the increase in the α-globulin components here observed will be discussed below in the light of the clinical findings.

Diagrams typical for this second group of sera are shown in Fig. 2.

In the third group (C) of sera examined and compiled in Table III, the common denominator is a significant increase in the relative concentration of the γ-globulin fraction. The albumin-globulin ratio is below unity with one exception, Experiment 459.

TABLE II. ELECTROPHORETIC COMPOSITION OF SERA FROM LYMPHOGRANULOMATOUS PATIENTS IN GROUP B; IN THIS GROUP THE RELATIVE CONCENTRATION OF EITHER ONE OR BOTH ALPHA GLOBULIN COMPONENTS IS INCREASED AND THE A/G RATIO IS BELOW UNITY

EXPERIMENT	AGE (YR.)	SEX	DURATION OF ILLNESS, ONSET TO EXPERIMENT	NUTRITION AND CLINICAL STATUS	A/G RATIO	RELATIVE CONCENTRATION IN PER CENT OF TOTAL AREA				
							GLOBULINS			
						ALBUMIN	ALPHA-1	ALPHA-2	BETA	GAMMA
373	37	M	17 mo.	Poor; Terminal	0.91	47.7	4.7	14.8	16.2	16.5
378	47	M	14 mo.	Poor; Terminal	0.73	42.1	10.4	13.7	15.3	18.5
381*	39	F	5 mo.	Poor; Terminal	0.46	31.6	15.4	19.0	20.5	13.5
382*	38	F	19 mo.	Poor; Terminal	0.67	39.5	11.7	13.4	22.3	13.0
382*	38	F	19 mo.	Poor; Terminal	0.66	40.0	12.7	12.9	22.0	12.5
419	27	M	21 mo.	Poor; Terminal	0.52	35.0	18.1	17.0	20.2	9.77
420	22	M	30 mo.	Poor; Terminal	0.55	35.4	14.0	17.9	12.8	19.9
430	56	F	21 mo.	Poor; Terminal	0.55	35.7	18.3	21.5	11.4	13.2
438	43	F	5 mo.	Fair; Terminal	0.49	32.7	13.4	20.3	14.6	18.9
463	61	F	6 wk.	Poor; Terminal	0.36	26.5	15.7	23.0	17.0	17.5
466	24	M	21 mo.	Poor; Terminal	0.54	35.2	20.6	19.3	10.3	14.5
Mean value					0.59	36.5	14.1	17.5	16.6	15.3
Average deviation					±.16	±4.2	±3.3	±2.9	±3.5	±2.7

*Areas calculated from ascending boundaries.

TABLE III. ELECTROPHORETIC COMPOSITION OF SERA FROM LYMPHOGRANULOMATOUS PATIENTS IN GROUP C; IN THIS GROUP THE RELATIVE CONCENTRATION OF GAMMA GLOBULIN IS INCREASED WHILE THE A/G RATIO IS, AS A RULE, BELOW UNITS

EXPERIMENT	AGE (YR.)	SEX	DURATION OF ILLNESS, ONSET TO EXPERIMENT	NUTRITION AND CLINICAL STATUS	A/G RATIO	RELATIVE CONCENTRATION IN PER CENT OF TOTAL AREA				
							GLOBULINS			
						ALBUMIN	ALPHA-1	ALPHA-2	BETA	GAMMA
368	24	M	2 yr.	Fair	0.83	45.5	5.3	10.3	12.2	26.7*
442	19	F	1 yr.	Good	0.61	38.2	8.9	13.2	13.4	26.2
444	51	M	3 yr.	Fair	0.67	40.4	6.8	13.0	14.5	25.4
429-b	33	M	27 mo.	Good	1.07	56.5	3.6	9.2	14.0	16.7
429-c	33	M	28 mo.	Good	0.95	48.8	8.9	12.7	10.2	19.5
460-a	26	F	1 yr.	Good	0.58	36.6	10.4	13.7	11.1	28.2
460-b	26	F	13 mo.	Good	0.68	40.3	10.3	13.6	10.7	25.1
461	20	F	4 yr.	Good	0.88	46.7	8.8	13.8	11.3	19.4
462	43	F	5 mo.	Good	0.57	36.3	9.6	16.3	14.9	22.9
480-b	19	M	9 mo.	Good	0.71	41.6	9.5	14.5	13.1	21.4
Mean value					0.76	43.1	8.2	13.0	12.5	23.2
Average deviation					±.14	±5.0	±1.8	±1.4	±1.4	±3.2

*Gamma globulin plus x component.

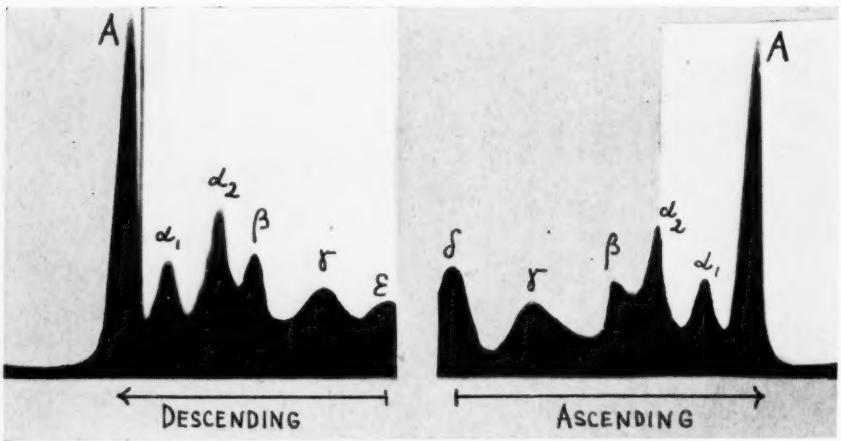

Fig. 2.—Electrophoretic diagram of serum from a patient with Hodgkin's disease, falling into the B group (Experiment 438, Table II): the pattern is characterized by relative decrease in albumin and corresponding increase in total globulin concentration (inverted albumin-globulin ratio) as well as by strikingly enlarged alpha-1 and alpha-2 globulin areas.

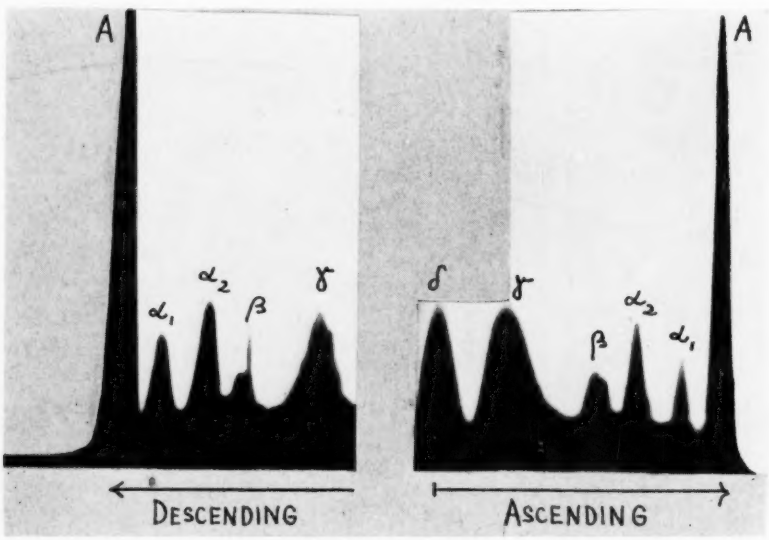

Fig. 3.—Electrophoretic diagram of serum from a patient with Hodgkin's disease, falling into the C group (Experiment 460-b, Table III): the pattern shows abnormally enlarged gamma globulin area in addition to increase in the alpha globulin components.

Experiments 429-b and 429-c were carried out on two blood samples from the same person, an interval of four weeks ensuing between collection of samples.

Diagrams of the Group C type are reproduced in Fig. 3.

In one instance, Experiment 368, an extra globulin component of an electrophoretic mobility intermediate between that of gamma and beta globulin

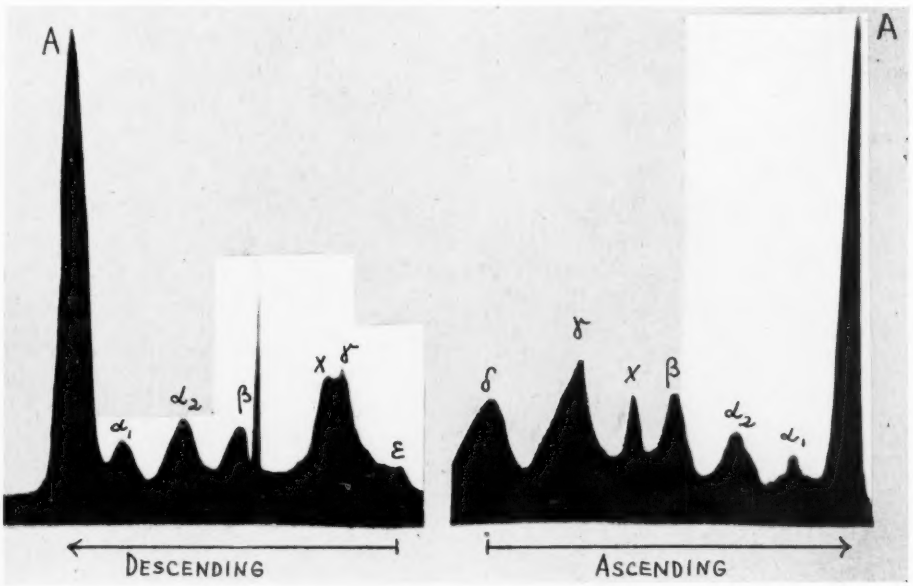

Fig. 4.—Electrophoretic diagram of serum from a patient with Hodgkin's disease, falling into the C group (Experiment 368, Table III): in addition to the increase in gamma globulin area, characteristic for this group, the pattern also shows the presence of an additional protein component, labeled x on the diagram, with a mobility intermediate between beta and gamma globulin. The x and gamma globulin maxima are fully resolved only in the ascending limb of the cell.

was observed (see Fig. 4). This component, labelled x in the diagram, was fully resolved from the γ-globulin boundary only in the ascending limb of the cell.

Comparison of the tables will show that the distinction between the three groups of sera is not too sharply defined in all instances. As would be expected, sera of intermediate type were encountered in this study, rendering their classification difficult and somewhat arbitrary. This indicates the existence of transitions from one group to another which, perhaps, reflect transitory changes in the clinical state of the patient (see below). Thus, the specimen examined in Experiment 429-b might also be placed in the A group, while the specimen from the same patient used in Experiment 429-c clearly falls in the C group. Other instances are the sera studied in Experiments 460-a and 460-b, which show increases in the relative concentrations of the alpha as well as of the gamma globulin fractions, and those analyzed in Experiments 373 and 457.

CLINICAL FINDINGS

Group A.—The thirteen sera in Table I were obtained from eleven patients. All were ambulatory, in a good nutritional state, and free from such symptoms of Hodgkin's disease as noticeable enlargement of lymph nodes. Experiments 467-a and 467-b were performed on specimens taken from the same patient

at an interval of one month, while Experiments 443-a and 443-b were done on the serum of another patient with an intervening period of four months. Since in one patient (Experiment 467-a) the disease had been manifest for only six months and in another (Experiment 422) for five years, the duration of the disease does not seem, of itself, to be responsible for the shape of the electrophoretic pattern. All patients in Group A had at one time or another received x-ray therapy; two of the patients were under x-ray treatment at the time the serum was withdrawn from them. With the exception of one patient (Experiment 422), who died ten months after his serum was examined, all patients are alive at the present time. Four of them have not required further therapy in the intervening period; their red cell sedimentation rates are normal or only slightly increased above normal value. Five other patients (Experiments 429, 457, 467, 480 and 483) have since been treated with mustard gas following enlargement of the lymph nodes, recurrence of pain, and loss of weight. In all instances the response to the treatment has been satisfactory.

Group B.—In striking contrast to the sera in Group A, the ten sera falling into Group B were obtained from patients in an advanced stage of Hodgkin's disease, terminal cases actually. The patients were very ill at the time the specimens were obtained. They were bedridden and emaciated and had fever. All the patients in this group died within three days to five months after the blood samples were withdrawn for electrophoretic study.

Group C.—More than one electrophoretic experiment was performed on the serum of three of these patients. Experiments 460-a and 460-b were carried out on the serum of the same patient with an interval of one month, Experiments 480-a and 480-b on the serum of another patient with an interval of two months (Experiment 480-a will be found in Table I). In a third instance of multiple analysis the first experiment fell into Group A (see Experiment 429-a, Table I), while subsequent experiments on serum samples withdrawn from the same patient after three and four months (Experiments 429-b and 429-c, respectively) were classified as Group C patterns (see Table III). Actually the diagram obtained in Experiment 429-b represents a borderline case between Group A and C sera. In neither of these patients were we able to discover a clinical basis for these variations in the electrophoretic pattern.

All of the patients in this group were ambulatory and in good nutritional state. Considered as a group, however, they represented more severe states of Hodgkin's disease than those appearing in Group A; they suffered more from fatigue (particularly patients furnishing the serum specimens for Experiments 461, 462, and 460-b). The patient represented by the sample in Experiment 368 exhibited recurrent enlargement of lymph nodes so severe as to require the institution of x-ray therapy. Two of the patients in Group C have died (Experiments 318 and 462). One other patient (Experiment 442) has been bedridden nine months and is at the present time in a terminal stage of the disease.

Attempts to correlate the electrophoretic patterns recorded in this study with individual factors, such as duration of illness, x-ray therapy, anemia, age of the patient, or sex, failed. However, there is an indication that the Group B pattern prevailed in those instances in which the disease ran a rapidly fatal course. Five of the sera in this group (B) were obtained from patients who had had Hodgkin's disease less than a year, while the remaining five had been ill for about one year. This is in contrast to the patients represented by Table I, five of whom had been ill for less than a year, three for a year, three for two years, and one for three to five years. Group C shows a more general distribution as to disease duration, since two each had been ill for less than one year, for one year, and for two years and one each for three and five years.

DISCUSSION

The observations made in this study indicate that the electrophoretic pattern of the serum proteins is nonspecific for Hodgkin's disease. Thus none of the thirty-three analyses made by us produced a pattern peculiar to Hodgkin's disease. Three types of curves—designated A, B, and C—were, however, obtained, and each curve could be more or less correlated with the clinical state of the patient. Thus the A curve was obtained from patients whose nutritional state was, and is to date, normal. The B curve, characterized by elevated alpha globulin components, was produced by terminal stage patients, those who were cachectic, anemic, febrile, and edematous. Finally, the C curve, characterized by elevated gamma globulins, was furnished by patients in a stage intermediate between the two others, not terminal but definitely more advanced than that of patients in Class A.

Comparison of our findings with those obtained from sera of patients with tuberculosis failed to show any parallelism between tuberculosis and Hodgkin's disease. In the former disease the alpha globulin rose early in the course of the illness, while in the latter disease a rise of the same fraction occurred terminally. In tuberculosis[6] the increase in gamma globulin appeared to be related to immunologic processes while in Hodgkin's disease no such conclusion could be drawn.

Of further interest is the recorded increase of alpha globulin in pneumonia[7] and in other febrile diseases which are accompanied by tissue breakdown.[8]

In the course of our studies the question arose as to whether blood sera patterns were reversible from the B and C types to A. Only one example was found, Experiment 480-b, which fell into the Group C. A repeat test on a blood sample withdrawn from this patient one month later resulted in an A curve. The maintenance of a perfect nutritional state in this patient sixteen months after the tests despite recurrence of massive lymph node enlargement would indicate a relationship between nutritional state and the electrophoretic curve rather than a relationship between lymph node enlargement and the electrophoretic serum pattern.

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SPECIAL ANNOUNCEMENT

The following is inserted at the request of the Cutter Laboratories:

Berkeley, Calif.
May 6, 1948

For Immediate Release

Superseding our air mail release dated May 5, 1948 is the following statement from Dr. R. K. Cutter, president, of Cutter Laboratories. "Contamination has been found in another and entirely different glucose solution, dextrose 10 per cent in Ringer's, according to an announcement made today by Dr. R. K. Cutter, president, of Cutter Laboratories. The company is cooperating with the Food and Drug Administration, and is requesting the assistance of health departments throughout the country, in immediately recalling from hospitals Cutter's entire line of dextrose and other solutions for mass intravenous injection. Company officials believe that discovery of this new contamination makes questionable the use of any product produced in their intravenous solutions department until this entire contamination difficulty is solved. The other products produced in this department are concentrated dextrose, distilled water, sodium citrate, normal saline solutions in 50 and 100 c.c. bottles, as well as all flasks supplied by Cutter for community blood and plasma banks.

"The reason for this contamination is still unknown, and until they have the positive answer, Cutter feels this is the only step that can be taken in the interest of public safety. In the meantime, arrangements are being made to supply hospitals with solutions of other manufacturers."

Cutter Laboratories.

THE TISSUE DISTRIBUTION OF RADIOANTIMONY INHALED AS STIBINE

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INTRODUCTION

RECENT problems in the field of tropical diseases have served to emphasize our present limitations in their treatment. To further the therapeutic use of compounds of antimony in these diseases, a better knowledge of the physiologic and pharmacologic properties of the element is necessary. New and more precise methods of assay for antimony have been recently developed which can be used for studies in vitro and in vivo.[1, 2]

Since the toxicity of a drug is, in general, a function of both concentration and time of retention, a given agent is likely to show varying toxicities to different organ systems of the host and parasite depending upon the extent and chemical state of local accumulation and the rate at which it occurs.

Inhalation of a gaseous compound of antimony, stibine (SbH_3), has been reported effective in reducing the parasite count in chicks infected with *Plasmodium gallinaceum*.[3] It therefore seemed of interest to learn the concentration of antimony as a function of time in the blood and tissues of chicks (both normal and infected with *P. gallinaceum*) and in guinea pigs after treatment with stibine containing radioantimony.

METHODS

A radioactive tracer method was selected because it permitted rapid detection and accurate measurement of amounts as small as micrograms of the element per gram of tissue without the tedious chemical manipulations of microchemical procedures. Radioactive antimony is one of the more favorable elements for this purpose, since two relatively long half-life isotopes can be produced efficiently.

The antimony isotopes were prepared by the bombardment of antimony as a probe target in a sixty-inch cyclotron.* The following reactions were utilized.

$$51\ Sb^{121} + 1H^2 \longrightarrow 51\ Sb^{122} + 1H^1$$

$$51\ Sb^{123} + 1H^2 \longrightarrow 51\ Sb^{124} + 1H^1$$

The two isotopes have a half life of 2.8 and 60 days, respectively, and were used in the mixed form as produced.

Radioactive antimony was added to powdered magnesium in the ratio of 1:5 and fused in a furnace under N_2 to produce an alloy from which stibine could be generated at will by dropping a known amount of the alloy into a 50 per cent hydrochloric acid (HCl) solution in a special container.† The chamber used for exposing animals to various concentrations of the gas was designed to permit rapid withdrawal of gas samples and of exposed animals during the experiment.

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The opinions or assertions contained in this article are the private ones of the writers and are not to be construed as official or reflecting the views of the Navy Department or the Naval service at large.

Received for publication, Feb. 14, 1948.

*At the Department of Terrestrial Magnetism, Carnegie Institute of Washington.

†We are deeply indebted to Dr. S. H. Webster of the National Institute of Health, Bethesda, Md., for furnishing us with the basic details for this method of producing stibine.

Animals were removed from the chamber at the desired intervals, exsanguinated, and the various organs and tissues dissected and weighed immediately. Blood samples were at once centrifuged and the desired components taken up in 1 ml. Luer syringes. Organs and tissues were ground with small glass mortars and pestles and the samples measured by volume in Luer syringes. Occasionally the quantity of tissue was insufficient, so that organs for several animals were pooled. The measuring cups were of Lucite and were kept free of radioactive contamination as shown by check with Geiger counters. Each cup was used only once during any given experiment. A standard volume (0.4 ml.) of tissue or blood was used for measurement wherever possible. The radioactivity of the sample was measured by a beta ray counter at a standard distance. The conversion of counts per second to micrograms of antimony was made by reference to a standard sample containing a known amount of antimony, the radioactivity of which had been measured in a manner identical to that of the biologic samples.

PROCEDURES AND RESULTS

Four groups of from forty to seventy chickens (7 and 9 days old) and one set of twenty guinea pigs were gassed with stibine for a period of about fifty minutes, removed from the gassing chamber at the desired time, and sacrificed for analysis. Two groups of the chickens and the guinea pigs were from normal stock; a third group of chickens was found upon autopsy to have a respiratory infection, and the fourth had been heavily infected with *P. gallinaceum*. Parasite counts were made on each chicken in the fourth group prior to the gassing period. Stibine gas concentration for these experiments was about 25 parts per million.

Time-concentration curves were obtained for blood and certain of its fractions and for spleen, liver, kidney, heart, and brain. Each point in the curves is an average value for several individuals (two to six with the chicks, two with the guinea pigs). Some of the fluctuations observed are obviously due to sampling errors and the small number of animals used for each point. It is evident that the greatest variability occurs in muscle and, in general, the least in liver

TABLE I. ANTIMONY CONCENTRATION IN NORMAL CHICKS TREATED WITH STIBINE GIVING THE INDIVIDUAL DETERMINATIONS FOR ONE SERIES AND THEIR AVERAGES

TIME	TISSUE	MICROGRAMS ANTIMONY PER GRAM WET TISSUE	AVERAGE MICROGRAMS ANTIMONY PER GRAM WET TISSUE	STANDARD DEVIATION	TISSUE	MICROGRAMS ANTIMONY PER GRAM WET TISSUE	AVERAGE MICROGRAMS ANTIMONY PER GRAM WET TISSUE	STANDARD DEVIATION
0 min.	Heart	5.82, 6.63, 5.97	6.14	.56	Muscle	1.0, 1.9, 1.3	1.4	0.57
15 min.	Heart	6.81, 8.95, 7.40	7.72	1.41	Muscle	1.7, 2.6, 1.9	2.1	0.63
30 min.	Heart	7.44, 8.58, 7.69	7.99	0.68	Muscle	2.4, 2.6, 1.9	2.3	0.57
1 hr.	Heart	7.03, 6.15, 6.74	6.64	0.56	Muscle	2.0, 2.3, 1.2	1.8	0.74
2 hr.	Heart	6.08, 5.19, 5.52	5.60	0.56	Muscle	1.6, 1.6, 2.1	1.7	0.34
4 hr.	Heart	4.90, 4.16, 3.83	4.30	0.69	Muscle	1.3, 1.1, 1.5	1.3	0.24
8 hr.	Heart	3.66, 4.12	3.89	0.32	–	– – –	–	–
0 min.	Liver	19.7, 25.6, 29.1	24.8	5.84	Lung	12.4, 11.8, 12.8	12.3	0.63
15 min.	Liver	25.5, 25.8, 27.1	26.2	1.03	Lung	11.8, 15.5	13.6	2.62
30 min.	Liver	27.9, 28.5, 24.3	26.9	3.00	Lung	11.0, 9.3, 10.8	10.4	1.20
1 hr.	Liver	29.1, 30.1, 27.2	28.8	1.83	Lung	12.1, 9.2	10.7	2.02
2 hr.	Liver	22.3, 24.9, 23.7	23.7	1.50	Lung	9.8, 8.8, 7.6	8.7	1.32
4 hr.	Liver	26.3, 25.9, 24.3	25.5	1.35	Lung	5.4, 6.3, 5.8	5.8	0.51
8 hr.	Liver	23.2, 24.6	23.9	0.99	Lung	3.2, 3.5	3.4	0.67

(Table I). There are also other potential sources of variability, such as species differences and concurrent infections, but the characteristic curves of antimony concentration as a function of time are similar in comparable tissues.

The whole blood curves (Figs. 1 and 3) in all the experiments exhibit a high initial level which rapidly falls off, giving an exponential type of curve. One may note that the rate of disappearance of antimony from the chick blood is only about half as rapid as from the guinea pig blood.

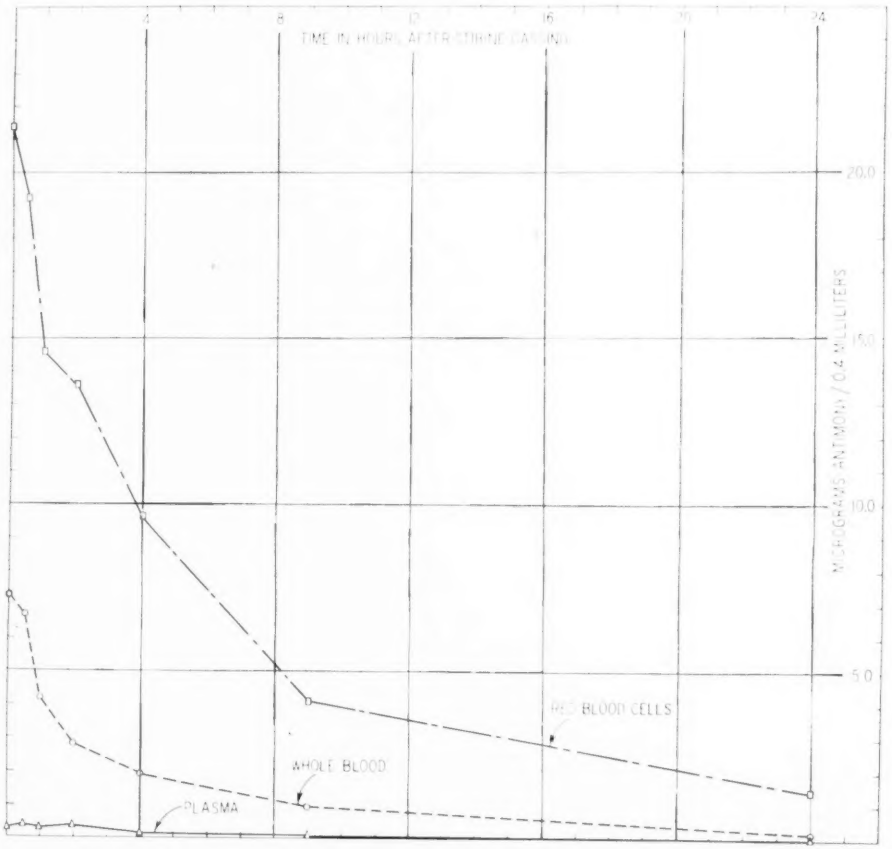

Fig. 1.—Antimony concentration in blood fractions of chicks infected with *P. gallinaceum*.

The curves of the blood fractions are essentially similar in character to those of the whole blood (Fig. 1). Although the red cell curve (Fig. 1) is not corrected for packing, it is still evident that initially the antimony is largely held within the red cells rather than in the plasma; later this partition is less evident and appears to approach a value of unity.

High doses of stibine are known to produce hemolysis of the red blood cells, and this occurred to some extent in these experiments. Both the direct hematocrit readings on the whole blood and the values obtained indirectly by calculations from the blood fraction levels of antimony showed a progressive fall in

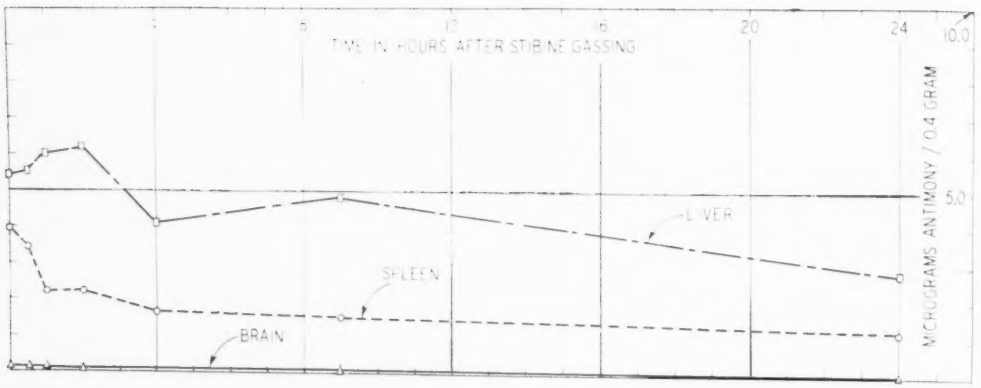

Fig. 2.—Antimony concentration in liver, spleen, and brain of chicks infected with *P. gallinaceum*.

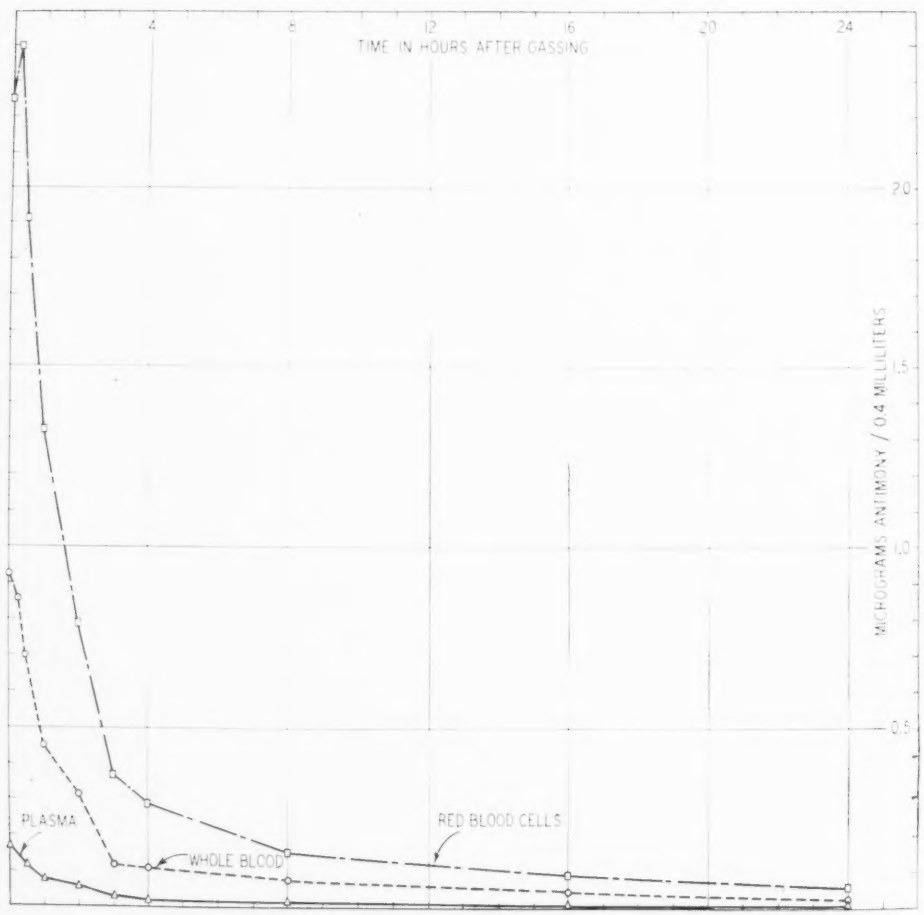

Fig. 3.—Antimony concentration in blood fractions of guinea pigs following stibine gassing. (Reproduced with the permission of the Naval Medical Bulletin.)

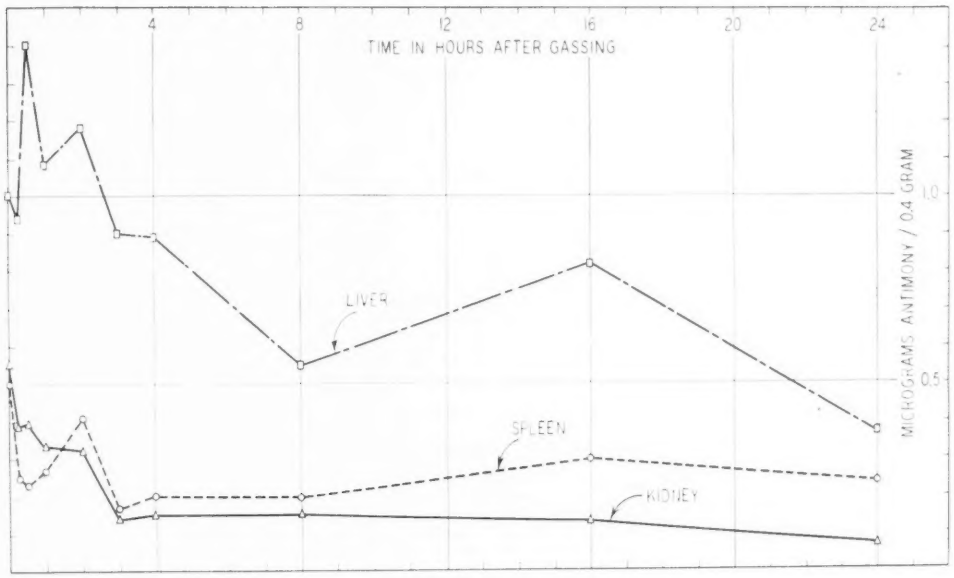

Fig. 4.—Antimony concentration in liver, spleen, and kidney of guinea pigs following stibine gassing.

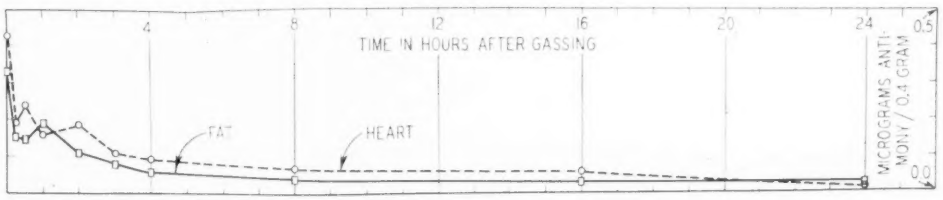

Fig. 5.—Antimony concentration in heart and perirenal fat of guinea pigs following stibine gassing.

total red cell volume during the first hour following gassing, amounting to about 30 per cent. After this the hematocrit remained constant at about 20 per cent red blood cells; whether the drop was due to hemolysis or not is uncertain.

From the present experiments the elimination curves of the lung, brain, muscle, and fat tissues (Figs. 2 and 5) appear similar to those of blood, and the ratio of the antimony content of these tissues to that of whole blood remains fairly constant during the course of the elimination of antimony from the body.

The concentration in other tissues follows a somewhat different curve, of which perhaps the most obvious example is that of the liver (Figs. 2 and 4). The result is the appearance of a maximum concentration, reached only after an hour or more. The spleen conforms in general to this same description. The curves for heart and kidney appear to fall in between the liver type and the blood type of exchange.

Some elementary insight into the dynamics of the biologic exchange of antimony may be gained from a study of the concentration ratio of antimony in the blood to that in the various tissues at successive times after the exposure.

This is illustrated by a time plot of the ratios of tissue to blood concentration for the guinea pig (Fig. 6). Data from the chick experiments yielded generally similar plots. Since the ordinate is logarithmic, it is easy to read the order of magnitude of the various ratios by inspection. For example, the liver concentration at four hours is eight times, while the plasma level is approximately one-fifth that of whole blood. The slopes of the curves also furnish evidence as to

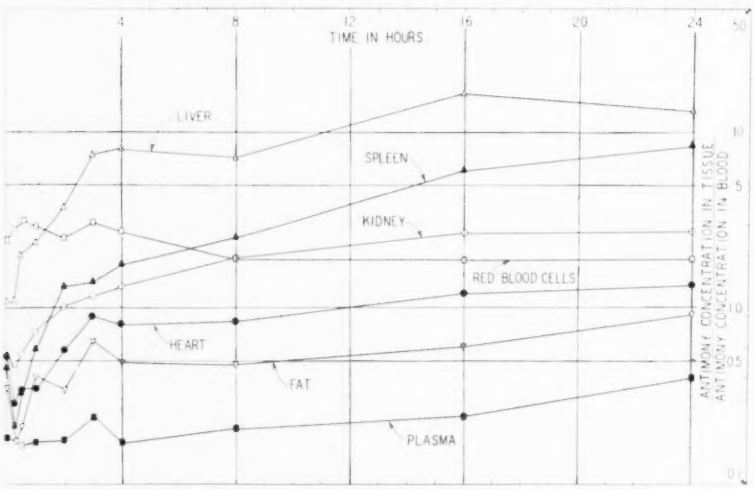

Fig. 6.—Ratios of antimony concentration in tissue to antimony concentration in blood (guinea pig) after stibine gassing.

whether the elimination from any given organ is proceeding at a rate equal to (slope zero), greater than (slope negative), or less than (slope positive) that of the blood. For most of the data the terminal slopes appear not to depart much from zero. This is further supported by data pooled from several experiments (Table II) showing that the antimony concentrations of each of these

TABLE II. AVERAGE PARTITION OF ANTIMONY BETWEEN TISSUES AND BLOOD IN CHICKS FOLLOWING TREATMENT WITH STIBINE; MEAN RATIOS (CONCENTRATION SB IN TISSUE/CONCENTRATION SB IN BLOOD)

TISSUE	HOURS AFTER GASSING								WEIGHTED GRAND AVERAGE
	8		16		24		52		
Liver	8.1	(4)*	12.0	(2)	9.1	(4)	7.4	(1)	9.11
Spleen	2.8	(3)	5.1	(2)	4.6	(3)	3.4	(1)	3.98
Kidney	2.1	(3)	1.7	(2)	1.9	(2)	1.6	(1)	1.89
Heart	0.64	(2)	0.85	(2)	0.90	(2)	0.65	(1)	0.776
Brain	0.017	(2)	0.014	(1)	0.018	(1)	----		0.017

*Number of experiments in which the tissue-blood ratio was measured.

tissues have attained a reasonably constant proportionality to blood at least by the end of eight hours.

With the foregoing in mind and with the knowledge that the blood curves (Figs. 1 and 3) exhibit a very rapid initial drop, it is clear that during the

initial period the blood is actually losing its antimony simultaneously to nearly all other tissues of the body and its observed concentration is more a function of total blood flow and relative vascularity of organs than of excretion. During the later stages the loss by excretion comprises a far greater percentage of the blood loss than is the case initially. Certainly beyond three or four hours it appears that the blood elimination becomes approximately equal to that of most of the tissues, and the concentration ratios remain essentially at constant levels, characteristic for different tissues. Thus, as the grand means in Table II indicate, after eight hours the highest concentration of antimony is found in the liver, followed by spleen, kidney, heart, and brain, in decreasing order. The concentration in the first three is always greater than in the blood; that in the heart is about equal to it and in the brain it is always about 1/100 of the whole blood level. A number of other tissues, such as muscle and fat, resemble brain in this respect and in their apparent tendency to approximate the plasma level of antimony concentration. This constancy in the tissue-blood ratios for the chick and the guinea pig may have certain therapeutic implications, since after eight hours one might estimate the probable tissue antimony concentration from that of the blood alone.

In both the guinea pig and chick experiments, the concentration of antimony in the bile was measured at the various time intervals and, in the guinea pig, that in the urine also. The curves for bile resembled those for liver, but the peak concentrations were ordinarily higher than for liver and required about twice as much time to develop. Thus the liver peak in the chicks was always reached at from one to two hours post gassing, while the biliary maximum occurred at around two and one-half to five hours. In the guinea pig the maximum in liver was at one-half hour and in the bile at two hours. The latter coincides with the time of the urinary maximum. Since in these experiments the total output of bile and urine was not known, quantitative estimates of excretion by these routes cannot be made. However, some information on the excretion of antimony, administered as stibine, may be obtained from the curve of the summated amounts in the various organs of the body as a function of time after the end of gassing, calculated from the concentration of antimony and the volume of the respective tissue. Such data yield curves which have been fitted by inspection (Fig. 7) and analyzed graphically. They give empirical equations expressing the elimination as a sum of decaying exponentials and their coefficients. Having obtained these expressions for the amounts present as a function of time, one may then differentiate to obtain the rate of elimination at any particular time. Thus, at the end of the gassing period the guinea pig is found to be excreting antimony at an initial hourly rate of around 60 per cent of the total amount present, while the chicks are much slower, ranging from 6 to 30 per cent per hour. Otherwise stated, it may be said that of the initial amount of antimony present at time zero, the guinea pigs had excreted 50 per cent by the end of the first forty-five minutes after gassing, while the chicks required from two to five hours to reach the same degree of elimination. The rough correspondence between the curve of elimination of the body as a whole

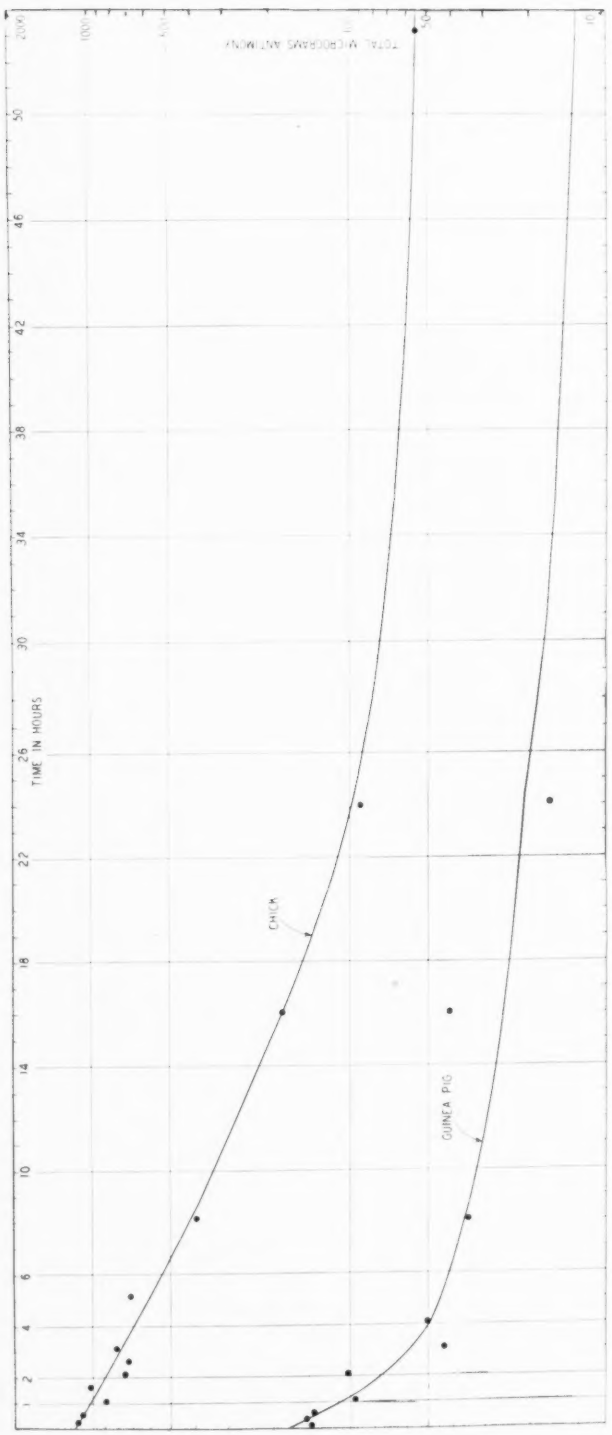

Fig. 7.—Total measured antimony content of body following stibine gassing.

(Fig. 7) and the blood curve (Figs. 1 and 3) appears to furnish additional evidence that, with stibine at least, a fair approximation to the elimination from the body may be had by recording the time curve of the blood after the first two hours have elapsed.

The single experiment in which chicks heavily infected with *P. gallinaceum* were gassed revealed no significant differences between these animals and the noninfected controls.

SUMMARY AND CONCLUSIONS

Radioactive antimony, administered as stibine gas (SbH_3) to chicks (both normal and infected with *P. gallinaceum*) and to normal guinea pigs, has been measured in the blood and tissues at successive time intervals following its administration. Significant differences were not apparent between distributions in normal and infected groups.

The concentration of antimony in the blood stream exhibited a smoothly decaying curve, decreasing more rapidly in the guinea pig than in the chick. The red cells contained initially a much higher concentration of antimony than did the plasma, although this difference was reduced with time.

The concentration curves of antimony in lung, brain, muscle, and fat were generally similar to those of blood, while those of the liver, and to a lesser extent of the spleen, passed through a maximum about one hour following treatment. Concentration curves of the kidney and heart were of variable shape.

Approximately four hours after treatment the tissue antimony levels became constant with respect to order of rank; those in the liver, spleen, and kidneys were greater than in whole blood; all other tissues showed concentrations less than the blood but as much as or more than the plasma.

Evidence is adduced to show that the rate of elimination from the body is higher for the guinea pig than for the chick.

In addition to the many who so generously assisted in the work from time to time, we wish to acknowledge with particular gratitude the assistance of L. H. Gordon, PhM2, V6, USNR, Roy L. Evans, PhM2, V6, USNR, F. N. Gillespie, PhM2, V6, USNR, and C. J. Spear, PhM1, V6, USNR.

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LABORATORY METHODS

A SIMPLE BEDSIDE METHOD FOR THE ESTIMATION OF BLOOD SUGAR

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THE reaction between glucose and dinitrosalicylic acid for the estimation of glucose was first used by Sumner in 1921.[1] Since then, the Sumner reagent has been in general use for the measurement of urine glucose and recently has been adapted for the determination of blood glucose on a tungstic acid filtrate.[2]

The following procedure is an extremely simple method for the approximate determination of blood sugar on a zinc hydroxide filtrate using the Sumner reagent as modified by Exton.[3] The method, although adaptable within certain limits for a strictly quantitative procedure, as discussed later, is primarily designed for an approximate estimation of blood sugar by the physician or nurse with a minimum of time and equipment and limited laboratory facilities. As shown in Table I, the approximate results obtained in five minutes using the color standards or color chart described here are essential checks with determinations as carried out by the Folin-Wu[4] macromethod and the Folin-Malmros[5] or the rapid Hagedorn-Halstrom-Jensen[6] micromethods.

REAGENTS

(A) 10 Per cent zinc sulfate ($ZnSO_4 \cdot 7H_2O$).

(B) 0.5 N Sodium hydroxide. (A stock solution preferably is kept in a paraffined bottle.)

These solutions, A and B, are the regularly employed macrosolutions for preparing a Somogyi blood filtrate.[7] They may be conveniently kept in small bottles provided with dropping pipettes graduated to deliver 0.4 ml. of solution. Stock bottles, tightly stoppered, keep well over a period of months.

(C) A solution of dinitrosalicylic acid prepared as follows:[3]

(1) A sodium potassium phenol stock solution is made by dissolving 400 Gm. of Rochelle salts (sodium potassium tartrate) in about 600 ml. of warm distilled water and adding 13 Gm. of phenol crystals. The Rochelle salts and the phenol are dissolved and mixed well, and the solution is then diluted to a volume of 1,000 milliliters.

(2) 12 Gm. of monosodium 3.5 dinitrosalicylate* are dissolved in 700 ml. of distilled water heated to a temperature of 65° C. When dissolved, the entire volume is poured into the liter of sodium-potassium phenol stock solution and mixed well. A yellow precipitate forms.

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Received for publication, Nov. 13, 1947.
*Eastman Kodak Company, Rochester, N. Y.

(3) 6 Gm. of sodium bisulfite ($NaHSO_3$) are added to the solution (2) and the whole is thoroughly mixed.

(4) Finally, 300 ml. of 10 per cent sodium hydroxide (2.5 N) are added and the final solution is completely mixed. The yellow precipitate formed in step 2 disappears at this point. The solution should be put in a brown bottle and allowed to stand for about one week before using.

To test the solution, titrate 5 ml. with normal acetic acid to the appearance of a white precipitate. If the reagent has been correctly prepared, 1.86 ml. of the acid should be required for 5 ml. of the reagent. This solution, if kept in the dark in a brown bottle, is stable for at least four to five months. If any cloudiness develops, the solution should be filtered before use.

APPARATUS

(A) A test tube approximately 12.5 cm. long and 15 mm. in diameter, graduated at 3.8 ml., Tube 1.

(B) A Pyrex test tube approximately 15 cm. long and 15 mm. in diameter, graduated at 2.0 and 4.0 ml., Tube 2.

(C) A cup or beaker in which a small amount of water may be boiled.

(D) A small funnel fitted with a piece of folded filter paper (Whatman No. 1, 5.5 or 7.0 cm. in diameter).

(E) A microblood pipette graduated at 0.1 and 0.2 milliliter.

(F) A prepared color chart or a series of color standards.

PROCEDURE

Measure into Tube 1, 0.4 ml. each of Reagents A and B, using the calibrated droppers with which the bottles are equipped. Add distilled water to the 3.8 ml. mark. In all quantitative measuring it should be remembered always to use just sufficient liquid to make the bottom of the meniscus on a line with the mark calibrated for the desired amount. Rotate the tube gently and introduce into the precipitating mixture exactly 0.2 ml. blood taken directly from the ear or finger by means of a micro blood pipette. (Venous blood may be used if collected with fluoride-oxalate* as a preservative and anticoagulant.) Mix the contents of the tube with the pipette by alternately sucking up and blowing out the blood mixture; then filter immediately into Tube 2 by means of the small funnel and filter paper. A water-clear filtrate should result.† Allow the filtrate to collect exactly to the 2 ml. mark. We have found that by using the technique described with Whatman No. 1 filter paper 5.5 or 7.0 cm. in diameter, 2 ml. of filtrate may be secured easily and rapidly. Add Reagent C to the 4 ml. mark. Mix thoroughly by rotation and immerse in a boiling water bath for exactly three minutes. The color changes during the heating period from a pure yellow to

*The fluoride-oxalate is prepared by thoroughly mixing together 50 Gm. powdered sodium fluoride and 20 Gm. powered potassium oxalate. Seventy milligrams of the mixture are used for each 5 ml. of blood.

†Although for complete precipitation of protein a freshly precipitated zinc hydroxide solution is not usually recommended unless heat is used, we have found that the double concentration of reagents yields a water-clear filtrate which gives an essentially negative biuret reaction.

TABLE I. COMPARISON OF BLOOD SUGAR VALUES, MG. PER 100 ML., BY DIFFERENT METHODS

SAMPLE	AUTHORS' RAPID METHOD		FOLIN-WU[4] (MACRO)	FOLIN-MALMROS[5] (MICRO)	HAGEDORN-HALSTROM-JENSEN[6] (MICRO)
	VISUAL	PHOTOELECTRIC			
1	300*			300	302
2	400*	404		400	
3	120		114		119
4	150	162	152	152	158
5	200	208		194	211
6	255	266	253	266	
7	220	225	218	228	224
8	250	258		248	252
9	125			129	122
10	100			105	90
11	290			278	289
12	100			97	94
13	200	208	200	198	
14	200		186		183
15	175	185	178		189
16	250*			244	
17	350			336	
18	50*			58	
19	200	208	191		193
20	225		195		
21	110				102
22	210		191		
23	160		157		
24	105			94	
25	110			105	
26	100			101	
27	110		97		
28	115			112	
29	102			94	
30	150	152	143	142	152
31	250	236	226	228	250
32	175	173			172
33	200	192		186	199
34	175	172	172		172
35	190	202	203	200	
36	105*	108		103	
37	580		630		
38	290†			270†	
39	265†			252†	
40	320†			338†	
41	190		178	180	
42	165		165		
43	155		141	148	
44	200†			206†	
45	100†			94†	
46	105†			92†	
47	100†			96†	
48	95		94		
49	105		102		
50	90		98		
51	110		114		
52	110	110	111	105	
53	220	225		218	228
54	390	374			376
55	100	87	90	85	
56	120	120	121	116	
57	100	97	90	85	
58	125	132	128	135	
59	110	101		103	
60	130	123		118	

*Comparison made with color chart alone.

†Different capillary blood samples obtained simultaneously from the ear.

a deeper yellow, yellow-brown, or reddish brown depending on the concentration of glucose present. Cool slightly after removal from the water bath and compare the color obtained with the color chart or standard tubes, matching it to the nearest color. Shades between standards can be approximated. Table I gives the results obtained on sixty blood samples using the described rapid estimation in comparison with other standard methods. In each case the rapid approximation was done either before the accurate determinations or was done by a second individual who had no knowledge of the correct results obtained with standard procedures.

PREPARATION OF COLOR STANDARDS

A series of accurate color standards may be prepared as outlined below. If prepared under the conditions given, these standards are accurate to within 5 per cent for a period of at least one month if kept in the refrigerator (40° F.) when not in use.

An accurate stock glucose solution prepared in saturated benzoic acid containing 0.2 Gm. glucose per 100 ml. is required. This solution is permanently stable if kept free from contamination. A dilute standard containing 10 ml. of this stock diluted to 100 ml. with distilled water is used in preparing the standard tubes as follows.

TABLE II. CHART FOR PREPARATION OF GLUCOSE STANDARDS

DILUTE GLUCOSE STANDARD (ML.)	BOILED WATER (ML.)	BLOOD SUGAR EQUIVALENT (MG. PER 100 ML.)
0.5	3.5	50
1.0	3.0	100
1.5	2.5	150
2.0	2.0	200
2.5	1.5	250
3.0	1.0	300

A series of tubes using quantities of the *dilute* glucose standard in increasing amounts of 0.5 ml. from 0.5 to 2.5 or 3 ml. is prepared. Freshly boiled distilled water which has been cooled is added in amounts to make a total volume of 4 milliliters. Then to each tube exactly 4 ml. of Reagent C are added; the contents of the tubes are mixed and the tubes placed in a boiling water bath for three minutes as outlined under Procedure for blood. After cooling, 4 ml. of the colored solutions are transferred to clean, dry tubes of the same diameter as those in which the determination is to be made. Two drops of toluol are added to each tube and the tubes stoppered and sealed with paraffin and labeled with the proper value in milligrams per 100 ml. of blood.

The series of standards which we have used is given in Table II. Standards between these values may be prepared but are not necessary. It is advisable, however, to prepare a new series of standard tubes with every new lot of Reagent C, as slight variations may occur.

Although the series of color standards may be preferable, a color chart from these standards may be prepared with ordinary paints. With a little experience good approximations can be made with the color chart. (Table I.) It was not feasible to reproduce here the color charts made and used by us.

DISCUSSION

The validity of the foregoing procedure as a rapid method for the estimation of blood sugar is apparent from the results given in Table I. Certain points regarding the method, however, should be noted. The reduction of the Sumner reagent, under the conditions outlined, is directly proportional to the concentration of glucose present between the equivalent of a blood sugar of 75 mg. and 300 mg. per cent, Fig. 1.

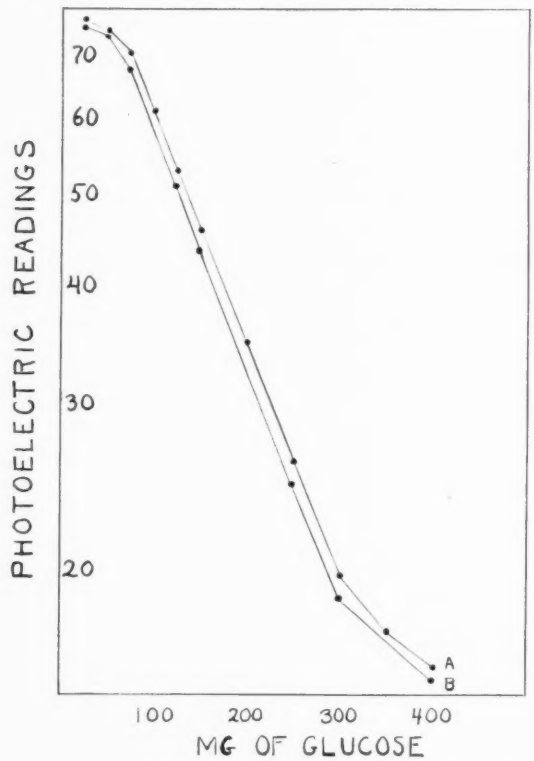

Fig. 1.—Two typical curves obtained from two series of standard glucose solutions using different lots of Reagent C. Curve A was done July 17, curve B, December 15. The readings were taken on a Leitz photoelectric colorimeter using the green filter No. 401 and plotted against the glucose concentration expressed as milligrams of blood sugar per 100 milliliters.

The curves given in Fig. 1 were obtained with standard glucose solutions using a Leitz photoelectric colorimeter with the green filter, Leitz No. 401. Slight variations occur, as indicated by these two typical curves, with different lots of Reagent C. It is essential, therefore, if the color of an unknown is to be measured accurately on a previously standardized photoelectric colorimeter, that the instrument be restandardized with each new lot of Reagent C. Although quanitative technique throughout the procedure would obviously be desirable if the final measurement of color was exact, our results do not warrant such an assumption as a necessity. With accurately calibrated tubes and reasonably careful technique, any error due to lack of quantitative pipetting is not of clinical significance, Table III.

TABLE III. COMPARISON OF BLOOD SUGAR VALUES WITH AND WITHOUT QUANTITATIVE MEASUREMENT, MG. PER 100 ML.

SAMPLE	TECHNIQUE AS RECOMMENDED		QUANTITATIVE MEASURING		FOLIN-MALMROS[5] OR FOLIN-WU[4]
	VISUAL	PHOTOELECTRIC	VISUAL	PHOTOELECTRIC	
1	110	110	100	100	105
2	120	120	110	117	116
3	100	97	105	105	90
4	125	132	120	130	135
5	150		150	157	150
6	200		200	213	202
7	150		150	163	160

In the procedure as recommended, the precipitation of blood protein is effected by means of freshly precipitated zinc hydroxide. The concentration of zinc sulfate and sodium hydroxide used is sufficient to yield a protein-free filtrate without the use of heat.* The filtration is rapid and the necessary volume of filtrate is quickly and easily obtained. A second procedure for precipitation of the protein may be used if desired. The blood may be pipetted directly into 3 ml. of water, the pipette being rinsed thoroughly by alternate sucking up and blowing out of the water solution. To the blood and water solution, then, the necessary quantities of Reagents A and B are added, the tube is stoppered, and the contents are mixed by shaking well. Using this method for precipitation of the protein, filtration is less rapid and the total volume of filtrate is slightly less. If this method of precipitation is adopted, it is recommended that tubes graduated at 1.5 and 3.0 ml. be used, rather than at 2.0 and 4.0 milliliters.

TABLE IV. COMPARISON OF BLOOD SUGAR VALUES OBTAINED WITH DIFFERENT TECHNIQUES FOR PRECIPITATION OF PROTEIN, MG. PER 100 ML.

SAMPLE	TECHNIQUE A*		TECHNIQUE B†		FOLIN-MALMROS[5] OR FOLIN-WU[4]
	VISUAL	PHOTOELECTRIC	VISUAL	PHOTOELECTRIC	
1	100	90	75	76	81
2	115	109	115	109	101
3	110	111	95	87	105
4	95	100	80	77	85
5	120	123	110	125	112
6	220	224	200	187	218
7	390	374	385	366	376
8	105	104	95	93	99

*Blood introduced into $Zn(OH)_2$.

†Blood introduced into water, then $ZnSO_4$ solution (Reagent A) added followed by NaOH solution (Reagent B).

This method for precipitation of the protein offers the advantage of not getting the protein precipitate in the micropipette, thus making the cleaning of the pipette considerably easier. Furthermore, this method for precipitation of the protein yields a true Somogyi filtrate and in some instances the blood sugar values obtained may be appreciably lower (Table IV), approaching probably the true blood sugar value.

Since, in its simplest form, the method is dependent for the final estimation on a color comparison with standard tubes or a color chart, the importance of

*See footnote †, p. 645.

standardizing one's individual technique for judging the color cannot be overemphasized. If standard tubes are used, the unknown solution should be in the same size tube as the standards. In using the standard tubes, we have obtained the best results by holding the unknown tube between the two closest standards and judging the light which comes through the tubes. In this way the density of the color can be fairly accurately approximated. If a color chart is used, we have obtained the best results by holding the tube containing the unknown obliquely above the block of color nearest in shade, with the source of light coming toward the tube from behind the worker. We have used daylight for all of our comparisons.

The determination is designed for 0.2 ml. of blood. When a blood sugar value over 300 mg. per cent is found, greater accuracy can be obtained by repeating the determination using 0.1 ml. of blood and subsequently multiplying the result by two. In this way, comparison is made with a standard under 300 mg. rather than by an attempt at a comparison with the very deep red-brown colors obtained with a 400 or 500 mg. standard.

The method in its present form is not suitable for the estimation of very low blood sugar values. Between 50 and 75 mg. per cent, fairly good approximations can be made. Below 50 mg. per cent the color of the reagent blank is so intense that any increase of color due to glucose is indiscernible.

SUMMARY

A simple method designed for the rapid estimation of blood sugar by the physician or worker with a minimum of laboratory experience and equipment has been described. The method is based on the reduction by glucose of dinitrosalicylic acid and closely checks determinations done by other standard methods.

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Erratum

In the paper by Maltaner and Gnesh, A Method for the Determination of Titers Between 10 and 100 in the Quantitative Complement Fixation Test for Syphilis, which appeared in the March issue of the JOURNAL (**33**: 383, 1948) in the column heading of Table II, p. 384, 12.0 should read 12.5.